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STUDIES UPON THE TIME OF FLOWERING OF PLANTS

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I. THE RELATION OF NOCTURNAL TRANSLOCATION TO THE TIME OF FLOWERING

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(With 20 Text-figures)

THE work of Garner & Allard (1920, 1923, 1925, 1931) on the response of certain plants to the length of day is, perhaps, one of the most illuminating pieces of botanical research during recent times. Three types of response have been demonstrated, namely, "long-day plants", which flower in the long days of summer, "short-day plants", which remain vegetative during summer and flower in the short days of autumn, and an indifferent type, which flowers at variable periods. It cannot be claimed that a complete explanation of the phenomenon has been advanced, though the original investigators, with C. W. Bacon, performed extensive determinations of hydrogen-ion concentration and water content (Garner *et al.* 1924). An announcement in the original paper (Garner & Allard, 1920), since elaborated by further experiment (Garner & Allard, 1931), seemed to have particular significance; Garner & Allard studied the effect of breaking the continuity of the daily illumination. They found that when plants were placed in the dark for periods of 1-5 hr. in the middle of a long day, the reproductive activities were not markedly changed, as compared with the normal summer illumination. This applied to all short-day plants which were tested, and most long-day plants. Similar periods of darkness, when *continuous* with the natural night, hastened the time of flowering of short-day plants, but seemed in general to have little effect upon long-day plants. Any effect of shortened daylight upon summer-blooming plants was towards the postponement of flowering.

The working hypothesis which prompted the experiments reported in this paper was that a late-flowering or short-day plant did not begin any work of translocation or growth until after a period of several hours in the darkness of night. A long-day plant, on the other hand, would be

likely to commence translocation soon after the advent of night. The possibility of testing this apparent delay in nightly translocation seemed fraught only with the difficulties of a 24 hr. working day. Full collaboration of Mrs M. Grainger, M.Sc., has, however, made it possible to follow the complete daily cycle of metabolism of thirty-four species of plants on ten occasions in 1935 and 1936. Mr G. Sheard, of Leeds University, has contributed material help with some of the determinations.

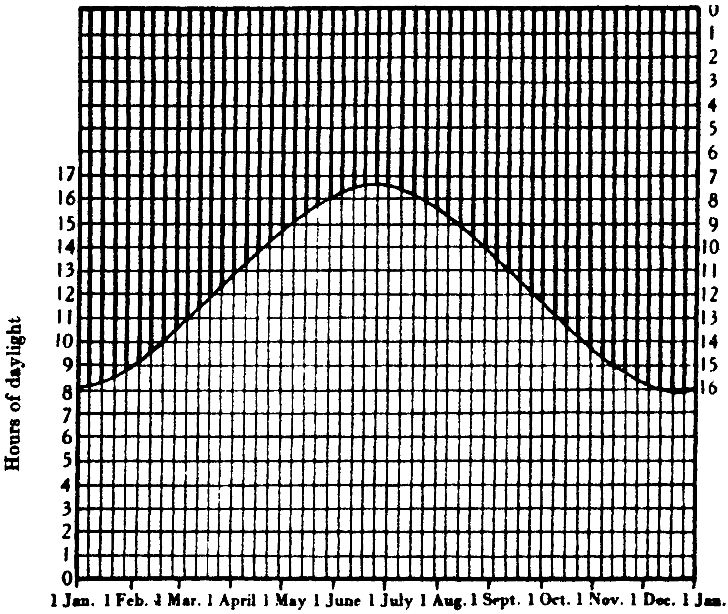

Fig. 1. Duration of daylight (light lines) and darkness (heavy lines) at Lat. 53° 40′ N.

The general plan was to investigate the carbohydrate metabolism of a large number of plant species, using qualitative determinations (section A). Two species were then selected for quantitative determinations, one a typical short-day plant, and the other a long-day species (section B). Several questions which arose as a result of these experiments were further tested by investigations of metabolism in prolonged darkness (section C), and by estimations of the rate of formation of starch when subsequently placed in daylight (section D).

The relative lengths of day and night for any time of the year are set forth in Fig. 1, which refers to the latitude of Huddersfield, 53° 40′ N.

Duration of the period of darkness for particular results is indicated by a thick black line above the diagrams (Figs. 2–14).

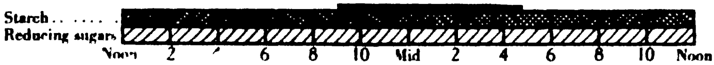

Fig. 2. Wallflower, 8–9 June 1935, vegetative; first-year plants. Similar metabolism was found in: Calceolaria, yellow flowered, 8–9 June 1935, vegetative. *Pteris cretica*, 8–9 June 1935, non-sporing. Stock, 22–23 July 1935, vegetative. *Jasminum nudiflorum*, 24–25 August 1935, vegetative. *Forsythia suspensa*, 24–25 August 1935, vegetative. *Populus canadensis*, 24–25 August 1935, vegetative. Rose—Polyanthus, 24–25 August 1935, flowering. *Tropaeolum*—Scarlet Gleam, 24–25 August 1935, flowering. *Mimulus*, 24–25 August 1935, flowering.

Fig. 3. Early flowering chrysanthemum, 20–21 June 1936, vegetative. Similar metabolism was found in: Antirrhinum, 4–5 May 1935, vegetative; reducing sugars present only from noon to 8 p.m.; 8–9 June 1935, vegetative; reducing sugars present only from noon to 8 p.m. Dahlia, var. Purple Robe, 8–9 June 1935, vegetative; reducing sugar present from 1 a.m. to 4 a.m. *Dianthus Allwoodii*, var. Freddie, 8–9 June 1935, vegetative; reducing sugar present from 1 a.m. to 5 a.m.

Fig. 4. *Sedum spectabile*, 22–23 July 1935, flowering. Similar metabolism was found in: *Sedum spectabile*, 9–10 April 1936, vegetative. *Reseda*, 22–23 July 1935, flowering; starch absent 3.30 a.m. to 5.30 a.m. *Primula variabilis*, 22–23 July 1935, vegetative; starch absent 10 p.m. to 10 a.m.; 20–21 June 1936, vegetative; starch absent 9 p.m. to midnight. *Primula wanda*, 9–10 April 1936, flowering; starch absent 1 a.m. to 9 a.m. *Vaccinium myrtilus*, 24–25 August 1935, vegetative; starch absent midnight to 6 a.m.

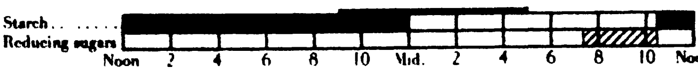

Fig. 5. *Clarkia elegans*, 22–23 July 1935, flowering.

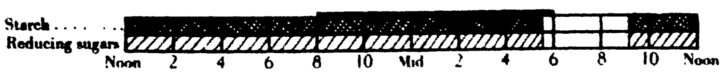

Fig. 6. Lettuce, 24–25 August 1935, vegetative.

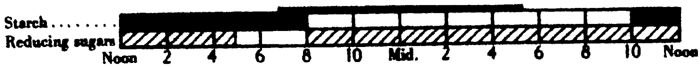

Fig. 7. Dandelion, 9–10 April 1936, vegetative.

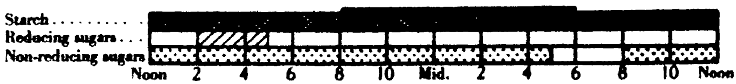

Fig. 8. Laburnum, 24–25 August 1935, vegetative.

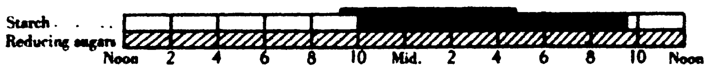

Fig. 9. *Limnanthes Douglasii*, 8–9 June 1935, flowering. Similar metabolism was found in: *Saxifraga tridactylites*, 8–9 June 1935, vegetative; starch present from 10.30 p.m. to 5.30 a.m.; 9 April 1936, flowering; starch present from 7 a.m. to 7 p.m.; 20–21 June 1936, vegetative; starch absent. *Saxifraga Aizoon*, 20–21 June 1936, vegetative; starch absent.

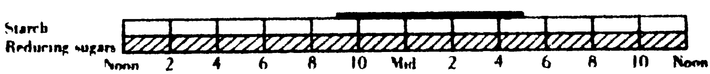

Fig. 10. Gladiolus, 22–23 July 1935, vegetative. Similar metabolism was found in: Auricula, 22–23 July 1935, vegetative, and Daffodil, 9–10 April 1936, flowering.

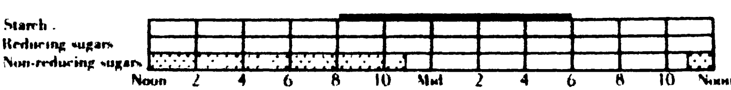

Fig. 11. *Holcus lanatus*, 24–25 August 1935, flowering. Similar metabolism was found in: *Poa annua*, 24–25 August 1935 and 9–10 April 1936; non-reducing sugars may be present throughout the whole 24 hr.

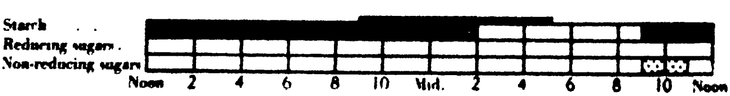

Fig. 12. *Cosmos bipinnatus*, 22–23 July 1935, vegetative.

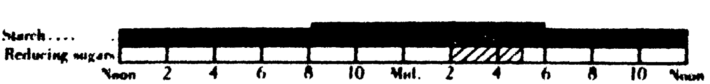

Fig. 13. Soy bean, 24–25 August 1935, vegetative.

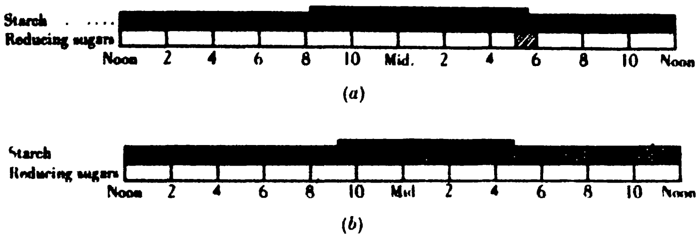

Fig. 14. Late-flowering chrysanthemum, var. Golden Seal: (*a*) in short daylight (long night), 27–28 April 1935; (*b*) in long daylight (short night), 8–9 June 1935.

A. Qualitative determinations

Material and experimental work

A wide variety of plants has been used for qualitative estimations for the presence or absence of starch and sugars. Their times of flowering ranged from February to October, and are set out in Table I. A sufficient number of plants of each species or variety was obtained, so that sample leaves could be taken without significant defoliation. Thus 144 antirrhinum plants were required, whilst fifty chrysanthemums and seventy dahlias sufficed for the experiments. Poplar, jasmine, forsythia, sedum, saxifrages, and some weed species were established in the writer's garden, and usually had sufficient branches or crowns that one or two leaves could be removed for each sample with impunity.

The supply of such numbers of plants would not have been easy, but for the valued co-operation of Miss J. Grainger, of Wilshaw, near Huddersfield, Mr T. Armstrong, Head Gardener of the Ravensknowle Park, Huddersfield, and Mr F. Crawshaw of Marsh, Huddersfield. These helpful gardeners propagated the plants at the appropriate times, and when the seedlings or cuttings were firmly established, they were transferred to the writer's garden at Dalton, Huddersfield, where all further growth took place. No samples were taken until the plants had been in their permanent quarters for at least a fortnight.

Qualitative determinations for the presence of starch were made by the ordinary Sach's test, and for sugars by Fehling's test. Leaves for the latter test were severed and treated immediately. Each was torn into fragments not more than 3 or 4 mm. square. These were introduced into a clean test-tube, and sufficient water to cover them was added. The amount of water in relation to the tissue was therefore roughly standard. This mixture was boiled for 2 min., when the clear liquid was decanted into another test-tube, and the Fehling's test performed for reducing

sugars in the usual way. A separate test for non-reducing sugars was made when required, hydrochloric acid being used for hydrolysis.

Table I

Plants used for the qualitative determinations

Scientific names cited without an authority for the species are interpreted in a horticultural sense. Figures in column 3 refer to the text-figures, which portray results of the experiments. The times of flowering are those observed locally, under the same conditions of climate and exposure as applied to the experiments reported in this paper.

Plant	Variety	Text-fig.	Time of flowering
Antirrhinum majus	Flame	3	Late July
Auricula, *Primula Auricula*	Chocolate-flowered	10	Mid April
Bilberry, *Vaccinium myrtilus*	-	4	May
Calceolaria rugosa	Bronze-flowered	2	Late July
Chrysanthemum, early-flowering	Sunburst	3	Late July
Chrysanthemum, late-flowering	Golden Seal	14	October
Clarkia elegans	-	5	July
Cosmos bipinnatus	—	12	Late July and August
Daffodil	Spring Glory	10	Mid April
Dahlia	Purple Robe	3	July
Dandelion, *Taraxacum officinale* Wigg.	—	7	May
Dianthus Allwoodii	Freddie	3	First week in July
Forsythia suspensa	—	2	February
Gladiolus	—	10	September
Holcus lanatus L.	-	11	August
Jasminum nudiflorum	—	2	February
Laburnum vulgare		8	June
Lettuce, *Lactuca sativa*		6	July and August
Limnanthes Douglasii		9	Early June
Mignonette, *Reseda odorata*	-	4	July
Mimulus luteus	-	2	August
Nasturtium, *Tropaeolum majus*	Scarlet Gleam	2	July and August
Poa annua L.	—	11	Spring, summer and autumn
Populus canadensis	—	2	—
Primula variabilis	—	4	May
Primula wanda	—	4	April
Pteris cretica		2	Spored mid-July, but may spore almost continuously
Rose	Polyanthus	2	August
Saxifraga Aizoon	—	9	April
Saxifraga tridactylites		9	May and early June
Sedum spectabile	-	4	End of July
Soy bean, *Soya max*		13	Late September
Stock, *Matthiola* sp.	Summer-flowering	2	End of July
Wallflower, *Cheiranthus cheiri*	—	2	May and June

The method of extraction cannot be regarded as removing anything but the soluble matter which was free to move in the veins of the tissue, or which was leached out of damaged cells. Such a short period of boiling could not extract the soluble matter from within the cells of the relatively large pieces of leaf. These simple methods yield results of comparative value by virtue of the positive insight which they give into the nightly metabolism.

Samples were taken, and determinations made, approximately every 3 hr., throughout a period of 24 hr. The times of sampling were not always the same, but in the accompanying diagrams (Figs. 2–14 and 18–20) any change recorded thereon is represented arbitrarily as taking place midway between the times of the preceding and succeeding determinations. Thus in Fig. 3, for example, the time of disappearance of reducing sugar, namely 3 a.m., is inferred from a positive result of a Fehling's test at 1.30 a.m. and a negative result at 4.30 a.m. The very narrow band of reducing sugar in Fig. 14*a* is due to the fact that determinations were made at less than 3 hr. intervals in this particular case. The adoption of this arbitrary device should render the portrayal of the results by the figures sufficiently accurate, without the use of extensive tables.

Results

The diagrams illustrating the metabolism of long-day plants (Figs. 2–11) show that all species have potentially mobile carbohydrates. Reducing sugars are very soluble and easily transportable, and they are usually present all the time (Figs. 2, 4, 9, 10) or during a considerable part of the 24 hr. period (Figs. 3, 6, 7). Starch often disappears during the night (Figs. 4, 5, 6, 7), thus showing that there is a change from this insoluble carbohydrate, to a soluble and more transportable sugar. A few plants, e.g. *Holcus lanatus*, *Poa annua* and laburnum (Figs. 8, 11) show a diurnal periodicity of non-reducing sugar, which must be the transportable carbohydrate of these plants, instead of reducing sugar.

The short-day plants (Figs. 13, 14) are in marked contrast, for they have relatively immobile carbohydrates. No disappearance of starch can be shown, and in the short nights of summer no reducing sugar can be detected qualitatively (Fig. 14*b*). Only in the relatively long nights of April or late August has the presence of reducing sugar been detected immediately before dawn (Figs. 13, 14*a*). No periodicity of non-reducing sugar could be detected in either the late-flowering chrysanthemum or the soy bean.

This evidence upholds the working hypothesis mentioned above, that true late-flowering or short-day plants do not begin the work of translocation until several hours after the fall of darkness. Even in long nights, the production of a transportable sugar does not begin until a short time before dawn (Fig. 14*a*), and in the short nights of summer it may not occur upon a measurable scale. Starch formed in one period of sunlight has not been transported when the sun's rays again visit the earth. The amount of carbohydrate which is available for growth

activities is therefore limited, in comparison with long-day plants. Short-day plants are, relatively, starving for sugar in the midst of an abundance of immobile carbohydrate.

The attainment of a suitable ratio of carbohydrate to nitrogen has been advanced by Kraus & Kraybill (1918) and others as a cause of flowering, but the discovery of photoperiodic response by Garner & Allard seemed to indicate the presence of another factor. It would appear from the present results that a delay in the translocation of carbohydrate in short-day plants could delay flowering by virtue of its effect upon the carbohydrate-nitrogen ratio. The plant's supply of nitrogen from soil sources is probably maintained steadily throughout the summer, and is not likely to depend much upon relative length of day. Thus long-day and short-day plants would seem to have the same facilities for nitrogen intake, but the summer-blooming plants can put their carbohydrates into circulation, where short-day species have stores of unavailable starch and sugars—a "frozen currency" in the carbohydrate sense. A suitable carbohydrate-nitrogen ratio would therefore be attained quickly by long-day plants, and slowly by the short-day kinds, hence the delay in flowering of the latter.

It seemed that a possible explanation of the earlier flowering of short-day plants when summer nights are lengthened artificially, as mentioned above, might be found in the increased time for translocation afforded by the extended period of darkness. Experiments were designed to test this hypothesis, and are described in section C of this paper.

The number of early-flowering and summer-blooming plants used in these experiments is very much larger than that of late-flowering plants. Two only of the latter have been found with certainty, namely, late-flowering chrysanthemum and soy bean. A third species, *Cosmos bipinnatus*, was enumerated as a short-day plant by Garner & Allard, but its flowering period in Yorkshire often begins as early as the end of July. Other plants, raised from the same seed and grown in the same garden, may bloom as late as the end of September. Though it may be made to flower earlier by curtailing the length of day in summer, it is not such a typical short-day plant as the late-flowering chrysanthemum. The diagram of its carbohydrate metabolism (Fig. 12) also shows a disappearance of starch, and a diurnal periodicity of non-reducing sugar content. This is more typical of a long-day plant than a short-day species.

A number of plants flowering in the relatively short days of the early part of the year seem to have similar metabolism to the more typical long-day plants. Such are forsythia, jasmine, the primulas, daffodil,

auricula and the saxifrages. Most of these plants have some peculiarity of structure or of organization to account for their early blooming. The daffodil has a very specialized periodicity closely bound with its bulb habit and its monocotyledonous organization. Forsythia and jasmine are shrubs whose flower buds are made in the previous year; the primulas, auricula and the saxifrages have a similar habit. The saxifrages present an interesting case. Indications have been gleaned from the present experiments that these plants, whilst having in general a long-day metabolism, have a different detail of diurnal periodicity in the spring, from what appears in summer. This question, and that of climate in relation to daily metabolism, will be discussed in a separate paper.

B. QUANTITATIVE DETERMINATIONS

The cruder qualitative determinations must give place to more accurate estimations, once they have signified agreement with the working hypothesis. Experiments recorded above show that the early-flowering and late-flowering chrysanthemums would provide suitable material for comparison of the metabolism of long-day and short-day plants respectively. These species are similar, but the difference between their times of flowering is considerable, namely, between late July and early October. Samples were taken at approximately 3 hr. intervals during a substantial part of a 24 hr. period. Many more plants were, of course, needed than for qualitative work. Whole leaves were pulled from the shoot, and eight of them, taken from corresponding positions upon different plants, usually constituted the sample.

The fresh weight was obtained, each group of leaves was placed in a paper tray, and was then heated within a steam oven for 3 hr. All samples were removed, at the expiration of the 24 hr. period, to a drying oven at 65° C., where all remaining moisture was driven off. The dry weight was then obtained, the leaves were powdered in a mortar, and stored in glass tubes placed within a desiccator.

About 0·05–0·1 g. of the powdered tissue was weighed for analysis into a 3×1 in. glass tube. Alcohol (95 %) was then added at the rate of 10 c.c. per tenth of a gram of the sample, and the cold mixture was left for at least 36 hr. This method of extraction is very different from that employed in the qualitative work; it extracts all soluble matter, where simply boiling with water for 2 min. would only remove any soluble matter which was free to move in the leaf, or which was leached out from damaged cells.

The insoluble residue was filtered off with frequent washing, dried at 65° C., weighed, and then boiled with 3% sulphuric acid for 3 hr. It was then neutralized with sodium carbonate, filtered, washed with distilled water, and the filtrate made up to standard volume. Estimations on this liquid gave the content of insoluble carbohydrate. The alcoholic filtrate was made up to standard volume with the requisite quantity of 95% alcohol, and was estimated for soluble reducing sugars, and, when required, for non-reducing sugars.

Table II

Early-flowering chrysanthemum, 20–21 *June* 1936.
Sunset 9.20 *p.m.* *Sunrise* 4.42 *a.m.*

The figures after the percentages in the columns headed "Total carbohydrate", "Reducing sugars" and "Insoluble carbohydrate" represent the limits of error for the determinations, expressed as a percentage, upon the same basis as the respective results. Results enclosed within a heavy line are further portrayed by Figs. 15, 16.

Time of sampling	Dry matter % of fresh weight	Insoluble matter % of dry weight	Total carbohydrate % of dry weight	Soluble sugars: Non-reducing % of dry weight	Soluble sugars: Reducing % of dry weight	Insoluble carbohydrate % of dry weight
			Fully expanded leaves			
2.30 p.m.	19·20	84·70	51·27 ± 1·02	Trace	10·25 ± 0·508	40·34 ± 1·18
7.30 p.m.	20·90	80·61	58·38 ± 1·40	Trace	13·01 ± 0·645	44·94 ± 1·61
10.30 p.m.	19·35	74·92	45·14 ± 0·571	2·07	9·07 ± 0·571	34·00 ± 1·57
1.30 a.m.	19·18	64·22	43·86 ± 0·855	Trace	15·57 ± 0·857	28·00 ± 0·571
4.30 a.m.	18·25	71·25	37·02 ± 0·693	1·09	11·48 ± 0·594	24·55 ± 1·40
8.00 a.m.	18·29	67·90	32·56 ± 0·660	5·29	8·11 ± 0·377	19·06 ± 1·42
			Old leaves			
2.30 p.m.	16·33	74·42	32·56 ± 0·425	2·70	13·33 ± 0·283	16·53 ± 0·425
7.30 p.m.	13·24	63·06	32·31 ± 0·615	Trace	12·61 ± 0·615	18·77 ± 1·08
10.30 p.m.	14·65	74·61	40·16 ± 0·390	5·07	7·92 ± 0·130	27·17 ± 0·974
1.30 a.m.	11·54	77·00	42·60 ± 0·800	7·00	8·60 ± 0·400	27·00 ± 0·800
4.30 a.m.	12·24	75·00	39·42 ± 0·750	Trace	13·00 ± 0·750	25·55 ± 0·750
8.00 a.m.	12·99	72·45	37·68 + 0·870	Trace	16·81 ± 0·870	20·00 + 0·290

The picrate method of Willaman & Davidson (1924) was used to estimate the carbohydrates. This is convenient and accurate for comparison. It was found that the method gave reliable readings for total carbohydrates, and for reducing sugars, but discordant results were sometimes obtained for soluble non-reducing sugars. This would seem to be occasioned by the use of the picric acid as a hydrolysing agent, as recommended by Willaman & Davidson (1924). It has been mentioned by Davis *et al.* (1916) that certain hydrolysing acids destroy the fructose resulting from hydrolysis, and citric acid has been mentioned as an acid safe in this respect. Citric acid (10 and 20%), however, inhibits the

Table III

Late-flowering chrysanthemum, 20–21 *June* 1936.

Sunset 9.20 *p.m.* *Sunrise* 4.42 *a.m.*

The figures after the percentages in the columns headed "Total carbohydrate", "Reducing sugars" and "Insoluble carbohydrate" represent the limits of error for the determinations, expressed as a percentage, upon the same basis as the respective results. Results enclosed within a heavy line are further portrayed by Figs. 15–17.

Time of sampling	Dry matter % of fresh weight	Insoluble matter % of dry weight	Total carbohydrate % of dry weight	Soluble sugars: Non-reducing % of dry weight	Soluble sugars: Reducing % of dry weight	Insoluble carbohydrate % of dry weight
			Fully expanded leaves			
2.30 p.m.	16·20	78·78	31·67 ±0·444	3·53	9·66 ±0·303	18·48 ±1·06
7.30 p.m.	15·42	75·71	30·85 ±0·714	2·80	9·35 ±0·322	18·70 ±0·807
10.30 p.m.	15·89	82·77	31·18 ±0·515	Trace	10·13 ±0·267	20·19 ±0·533
1.30 a.m.	16·06	74·62	33·05 ±0·833	5·85	9·23 ±0·380	17·97 ±0·759
4.30 a.m.	14·86	79·98	32·05 ±0·972	6·90	7·68 ±0·316	17·47 ±0·526
8.00 a.m.	15·68	76·89	26·24 ±1·64	Trace	9·35 ±0·385	16·54 ±0·513
			Old leaves			
2.30 p.m.	13·31	84·85	29·05 ±0·625	2·56	8·65 ±0·471	17·64 ±0·471
7.30 p.m.	12·60	70·25	32·00 ±0·500	9·23	8·35 ±0·329	14·42 ±0·549
10.30 p.m.	13·72	68·02	34·65 ±1·16	6·21	13·88 ±0·695	14·58 ±0·695
1.30 a.m.	12·01	74·92	28·22 ±0·444	1·35	11·87 ±0·625	15·00 ±0·625
4.30 a.m.	12·29	74·20	34·43 ±1·47	4·74	14·54 ±0·605	15·15 ±0·605
8.00 a.m.	12·58	59·50	31·02 ±0·821	8·18	9·05 ±0·357	13·79 ±0·357

Table IV

Late-flowering chrysanthemum, 9–10 *April* 1936.

Sunset 6.45 *p.m.* *Sunrise* 5.20 *a.m.*

The figures after the percentages in the columns headed "Total carbohydrate", "Reducing sugars" and "Insoluble carbohydrate" represent the limits of error for the determinations, expressed as a percentage, upon the same basis as the respective results. Results enclosed within a heavy line are further portrayed by Fig. 17.

Time of sampling	Dry matter % of fresh weight	Insoluble matter % of dry weight	Total carbohydrate % of dry weight	Soluble sugars: Non-reducing % of dry weight	Soluble sugars: Reducing % of dry weight	Insoluble carbohydrate % of dry weight
			Fully expanded leaves			
6.30 p.m.	9·75	59·16	26·83 ±0·500	12·49	10·17 ±0·333	4·17 ±0·830
10.30 p.m.	9·15	59·23	23·30 ±0·539	8·31	10·92 ±0·385	4·07 ±0·769
2.30 a.m.	7·25	70·42	23·31 ±0·352	7·61	6·90 ±0·143	8·80 ±1·41
5.30 a.m.	8·34	62·22	16·22 ±0·555	4·22	5·89 ±0·222	5·11 ±1·11
8.30 a.m.	9·20	55·74	17·40 ±0·261	6·18	9·04 ±0·174	2·18 ±0·870
			Immature leaves			
6.30 p.m.	12·61	73·57	27·29 ±0·500	4·15	6·28 ±0·286	16·86 ±0·715
10.30 p.m.	12·10	73·04	14·63 ±0·522	3·65	8·78 ±0·267	2·30 ±0·609
2.30 a.m.	11·00	58·20	18·25 ±0·246	4·10	10·98 ±0·164	3·17 ±0·574
5.30 a.m.	10·87	68·75	12·95 ±0·535	4·11	7·05 ±0·268	1·79 ±0·446
8.30 a.m.	12·02	63·87	23·32 ±0·323	6·20	9·03 ±0·645	7·99 ±0·645

formation of the brown colour typical of picramic acid, and therefore nullifies the test. The chrysanthemum material seemed to respond quantitatively to hydrolysis by 3% sulphuric acid, as mentioned above, so the total carbohydrate was estimated by separate hydrolysis of an unextracted sample of the original powdered tissue. The figures under the columns headed "Total carbohydrate", "Reducing sugars" and "Insoluble carbohydrate" in Tables II, III and IV have therefore been estimated by direct analysis, whilst the figures in the column headed "Non-reducing sugars" have been obtained by difference.

Results

Tables II–IV and Figs. 15, 16 show that there is a delay in the translocation of carbohydrates from fully expanded leaves of late-

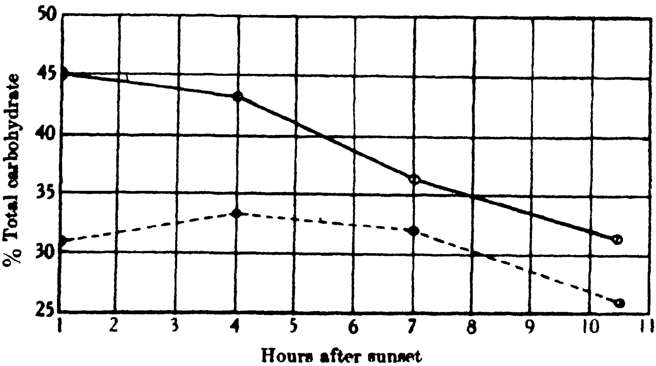

Fig. 15. Total carbohydrate content during the night of 20–21 June, 1936 (long-day conditions). Note the sustained and considerable reduction (13%) in the early-flowering chrysanthemum (——), compared with the slight (5%) and delayed reduction in the late-flowering kind (- - -).

flowering chrysanthemums, as compared with the early-flowering kind. It is not shown, however, by an increase in the amount of soluble reducing sugar as in the qualitative determinations, but by a decrease in the total amount of carbohydrate during the night. This is a surer criterion, and confirms the findings of the qualitative experiments, but from a different angle. Old leaves were also estimated for comparison, and showed that no substantial decrease in total carbohydrate took place during the night, in both early- and late-flowering chrysanthemums. What use are these old leaves to the plant? They transpire large quantities of water, their photosynthetic activity is at a minimum, and

they seem to act as unnecessary reservoirs for the storage of carbohydrates which could make a far greater contribution to the plant's growth if they were delivered at the growing-point. Young leaves, not more than 15 mm. long, showed a variable metabolism (Table V). It is interesting to note that leaves of the early-flowering chrysanthemum contain more carbohydrate at all times than the late-flowering kind.

Comparison of the behaviour of the late-flowering chrysanthemum in long-day illumination (Table III) with that under short-day conditions (Table IV) shows the relation of this delayed translocation to the relative length of night. Fig. 17 illustrates such a comparison; in June daylight appears before the fall assumes any substantial proportions; in April the

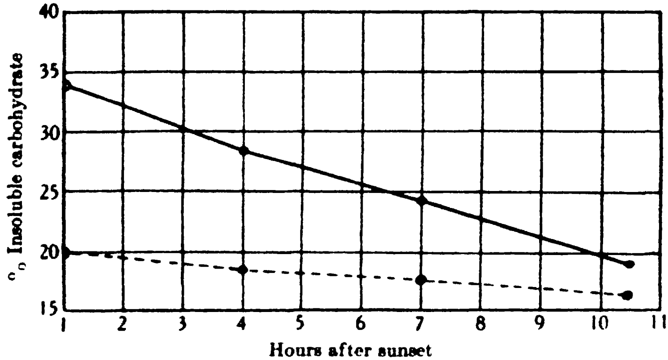

Fig. 16. Insoluble carbohydrate content during the night of 20–21 June 1936 (long-day conditions). Note the considerable reduction (15 %) in the early-flowering chrysanthemum (——), as compared with the slight reduction (4 %) in the late-flowering variety (- -).

fall occurs about 3 hr. before dawn. It is not to be expected that the content of total carbohydrate would rise immediately after dawn; there is a time lag before it begins to rise. A chrysanthemum plant takes a few hours to manufacture starch, even when previously kept in the dark, as is shown later in this paper. The lag follows a prolonged period of translocation in the short-day conditions, and only a short one during the long days of summer.

Table IV shows that no large increase in the amount of reducing sugar appears immediately before dawn, in April, in the late-flowering chrysanthemum, as would be suggested by the qualitative tests (Fig. 14*a*). This would be accounted for by the difference in methods of extraction, and the most likely inference is that the cells of late-flowering chrysanthemum leaves become more permeable a little time before dawn. They

would thereby allow more reducing sugar to leach out into the water used for extraction in the qualitative determinations. Extraction of *all* the soluble constituents by alcohol would not necessarily make any such discrimination.

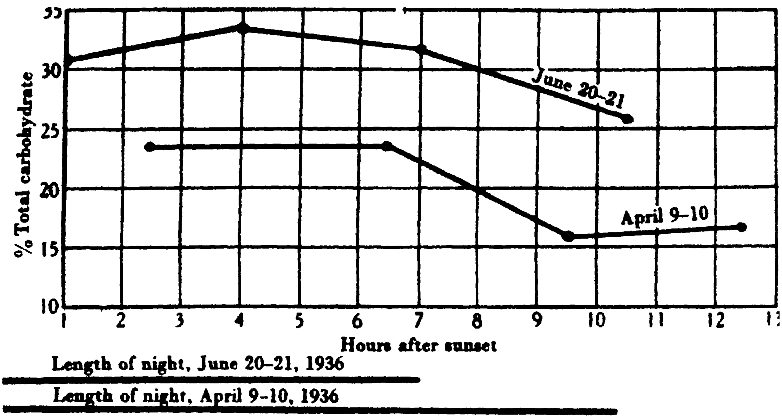

Fig. 17. Comparison of the total carbohydrate content of the late-flowering chrysanthemum, under long-day conditions (20–21 June) and short-day conditions (9–10 April).

C. Experiments with prolonged darkness

A repetition of Garner & Allard's original experiment upon the hastening of flowering of short-day plants by artificially lengthening the nights in summer was made at Weetwood, Leeds, in 1932. Late-flowering chrysanthemums of the varieties Golden Seal, Rayonanthe, Mrs N. Wells, Thora and Mauve Single, were raised from cuttings, and were grown in 10 in. pots in the usual way. They were subjected to a 12 hr. day, from 7 a.m. to 7 p.m. from 13 July, and by 25 August all varieties had bursting flower buds. Control plants, grown in normal daylight, did not flower until early October.

The qualitative experiments of section A show that reducing sugars can only be detected in the late-flowering chrysanthemum immediately before dawn, even in a relatively long night (Figs. 14*a*, *b*), and there is the above experimental evidence to show that flowering is hastened by lengthening the night artificially. Can reducing sugars be detected for a longer period during a night artificially lengthened? The following experiments suggest an answer in the affirmative.

Numerous plants of the late-flowering chrysanthemum, Golden Seal, were taken from the cool greenhouse of the writer's garden, and were

placed in a well-ventilated dark shed, in the early evening of 27 April 1935. Samples were tested for starch and reducing sugars at approximately 3 hr. intervals, until 4 p.m. of the following day. The results are given in Fig. 20, which shows that reducing sugars were present in detectable amounts for several hours. We now have the following facts:

(1) Late-flowering chrysanthemums, representing typical short-day plants, exhibit delayed translocation of carbohydrate through the night. During the long nights of April reducing sugar can be detected qualitatively only for a short time before dawn, and in the short nights of

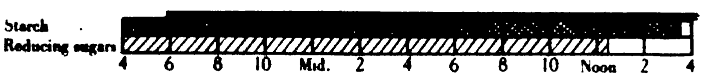

Fig. 18. Wallflower in prolonged darkness, 8–9 June 1935. Similar metabolism was found 8–9 June 1935 in: Calceolaria, Antirrhinum and Dahlia; but, in the two latter, reducing sugar did not persist long after the beginning of darkness.

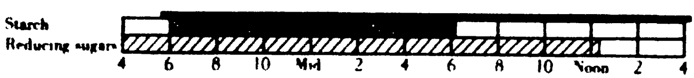

Fig. 19. *Limnanthes Douglasii* in prolonged darkness, 8–9 June 1935. Similar metabolism was found in *Saxifraga tridactylites*, 8–9 June 1935.

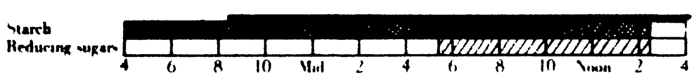

Fig. 20. Late-flowering chrysanthemum in prolonged darkness 8–9 June 1935.

summer it cannot be found at any time during a 24 hr. period. Such plants bloom naturally in the short days of late September or October.

(2) An artificial lengthening of the night in summer causes the reducing sugars to be detectable for a longer period, and at the same time makes the late-flowering chrysanthemum to flower in the relatively long days of August.

(3) Normal summer-blooming or long-day plants have reducing sugars detectable qualitatively for a considerable time during the 24 hr. period, or exhibit other evidence of the mobility of their carbohydrates.

The logical conclusion seems to be that an artificially lengthened night during summer causes a short-day plant to flower earlier than normal by virtue of the extended time for transportation of carbohydrate which it confers. This artificial treatment renders both the carbohydrate

metabolism, and the time of flowering, more like those of a long-day plant. A comparison of Figs. 18, 19 (long-day plants) with Fig. 20 will show this approaching similarity to long-day metabolism in extended darkness. When placed in such conditions, the late-flowering chrysanthemum loses its carbohydrates in about the same time as do such long-day plants as calceolaria and wallflower.

D. The rates of starch-formation in long-day and short-day plants

Some experiments kindly performed with the writer by Miss Enid Clegg, B.A., suggest that the late-flowering chrysanthemum can form starch quicker than some early-flowering species. Plants were kept in the dark for 35 hr. until all starch had disappeared from the leaves, and were then exposed to sunlight. At hourly intervals one plant was removed to the darkness, and at subsequent half-hourly intervals samples of its leaves were removed and tested for starch by Sach's test. The result of such an experiment, carried out on 3 October 1933, is given in Table V.

Table V

Late-flowering chrysanthemum plants deprived of starch, and then placed in sunlight, 9.15 *a.m.*, 3 *October* 1933

Brought from sunlight to darkness	a.m. 10.15	10.45	11.15	11.45	12.15	p.m. 12.45	1.15	1.45	2.15	2.45	3.15	3.45	4.15	4.45
A. At 10.15 a.m.	–	–	–	–	–		–		–					
B. ,, 11.15 a.m.	.	.	+	+	–									
C. ,, 12.15 p.m.	.	.	.		+	.					–			
D. ,, 1.15 p.m.	.	.	.		.	.	+	+	+	+	+	+	.	+
E. ,, 2.15 p.m.	.	.	.		.	.	.	.	+	+	+	+	+	+
Control (not exposed to sunlight)	–	–	–	–	–		–	–		–				

It will be seen that starch appeared after 2 hr. exposure to sunlight, but disappeared almost immediately after it was placed in the dark. After 4 hr. in the light, it took more than $3\frac{1}{2}$ hr. to disappear after its removal to darkness.

More definite results were obtained on 4 and 5 October:

Date	Length of time for starch formation hr.	Length of time for starch removal hr.
4 Oct	2	More than $3\frac{1}{2}$
5 Oct.	1	Between 2 and 3

It is noteworthy that it takes roughly twice as long to remove the starch as it does to make it.

Experiments on *Primula wanda*, an early-flowering plant, and *Sedum spectabile*, a long-day species, carried out on 19 April 1936 in the writer's garden, seem to show that these plants were slower to make starch, and quicker to lose it:

Plant	Length of time for starch formation hr.	Length of time for starch removal hr.
Primula wanda	More than 4	2
Sedum spectabile	,, 4	2

It may be noted here that it takes about half the length of time to remove the starch as it does to make it.

Table VI portrays the climatic features of the days upon which the above experiments were carried out, and the writer thanks Miss Ellen Gallwey, of the Ravensknowle Museum, Huddersfield, for the figures.

Table VI

Meteorological data for experiments on the rate of starch formation

Figures for temperature and humidity are corrected to the nearest whole number. Data for October 1933 refer to experiments carried out at the Tolson Memorial Museum, Ravensknowle, Huddersfield, where the meteorological station is situated. Data for April 1936 apply to experiments carried out at the writer's garden, approximately ¼ mile north of the meteorological station

	Temperature ° F.			Humidity %			Bright sunshine, hr.		Wind, m.p.h.		
Date	9 a.m.	3 p.m.	9 p.m.	9 a.m.	3 p.m.	9 p.m.	Sunrise–noon	Noon–sunset	9 a.m.	3 p.m.	9 p.m.
1933											
3 Oct.	48	55	42	86	69	98	—	0·4	—	—	—
4 Oct.	52	61	56	82	81	90	0·5	0·7	—	9·6	—
5 Oct.	56	58	54	85	76	93	—	—	—	—	—
1936											
19 April	42	45	40	51	52	75	6·6	4·8	14	12·8	5

These results seem to warrant the conclusion that the late-flowering chrysanthemum has, in addition to delayed translocation, the somewhat ironical incubus of facile starch production. We can now visualize this plant as having such a rapid photosynthesis that the leaves quickly become gorged with starch, which is not removed to any great extent during the night. *Primula wanda* and *Sedum spectabile* are relatively slow manufactories of starch, but it is steadily removed. The fable of the hare and the tortoise is most apt.

SUMMARY AND CONCLUSIONS

1. The late-flowering chrysanthemum, a representative of the "short-day" group of plants, showed evidence of delayed nocturnal translocation, when compared with a number of summer-blooming species. In the late-flowering chrysanthemum transportable carbohydrate did not appear

during the short nights of summer, and was only detectable shortly before dawn in the long nights of spring. Starch was always present in the leaves. Transportation in early-flowering plants could be inferred either from nocturnal disappearance of starch, or the abundance of reducing sugars, or a periodicity of non-reducing sugars. Quantitative determinations of various carbohydrate fractions of early-flowering and late-flowering chrysanthemum confirmed the delayed translocation in the latter, using the surer criterion of nightly decrease in the total and insoluble carbohydrates from mature leaves.

2. Artificial lengthening of the night during summer causes late-flowering plants to bloom earlier, and it is suggested that this effect results from the increased facility for translocation afforded by the longer period in darkness.

3. The results explain why a period of 4 hr. darkness around midday is not effective in hastening the flowering of a short-day plant (Garner & Allard, 1931). Such a period is not long enough for effective translocation to begin, and the plant has approximately as much carbohydrate at the end of the period as at the beginning.

4. Preliminary experiments indicate that the late-flowering chrysanthemum forms starch much more easily than two long-day plants which have been tested. The short-day species has a surfeit of starch and poor translocation; the long-day plants have slower manufacture of starch, which, however, is steadily removed. These results provide a necessary link between the hypothesis that a high carbohydrate-nitrogen ratio tends towards flowering, and the facts of photoperiodism. It is possible that delayed translocation of the late-flowering chrysanthemum postpones the attainment of a suitable ratio until the long nights of autumn, or artificially lengthened nights in summer, increase the nightly period of effective carbohydrate translocation, and thus the necessary balance is attained. A suitable carbohydrate-nitrogen ratio can readily be attained by long-day plants, by virtue of their effective translocation.

5. The results also focus attention upon the necessity of estimating the *mobility* of carbohydrate in considering its effect upon time of flowering. It is not sufficient to estimate the soluble carbohydrate, nor even the total carbohydrate, for neither of these figures, taken by itself, would give any adequate idea of the availability of the carbohydrate for altering the ratio with nitrogen.

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OBSERVATIONS OF THE EFFECT OF NITROGEN AND POTASSIUM ON THE FRUITING OF THE TOMATO

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(With 12 Text-figures)

CONTENTS

Introduction

Previous literature on the influence of nitrogen and potassium on the fruiting of the tomato includes communications from Bewley & White (1926), who described the symptoms of nitrogen and potassium deficiency, and Owen (1929, 1931), who investigated the chemical composition of the fruit and foliage. In America the effect of variation in nitrogen supply has been considered by Kraus & Kraybill (1918), Murneek (1925, 1926), Nightingale (1927), Nightingale *et al.* (1928) and Clark (1936) and that of potassium supply by Johnston & Hoagland (1929), Janssen & Bartholomew (1929), Nightingale *et al.* (1930) and Phillips *et al.* (1934). With the exception of the work of Murneek, to which special reference

is made below, the conclusions of these investigators that are germane to the present observations are referred to as occasion arises in the presentation of the results.

Murneek (1926) concludes that there is a negative correlation between vegetative activity and fruiting. A plant carrying a heavy crop receives a check to vegetative development which continues until the crop has matured, a recurrence of this process leading to "cyclic growth" (1925). Murneek (1926) claims that the presence of one fruit only on a nitrogen-starved plant is sufficient to check further vegetative extension, and attributes this effect to the monopolization by the fruit of practically all the nitrogen absorbed and elaborated, combined with the inability of the tomato plant to store appreciable amounts of nitrogen. "It is evident, therefore, that *a condition of nitrogen starvation with all its attendant manifestations can be brought about in vegetative parts of the tomato by the correlative effects of the fruit, and quite independently of the external supply of nitrogenous nutrients*" (Murneek, 1926, p. 26). Murneek supports his views with some striking analyses. He shows that with abundant nitrogen supply 46 % of the total nitrogen of the plant is contained in the fruits,[1] so that in defruited plants the nitrogen content is raised from 29 to 57 % in the leaves, 14 to 30 % in the stems, and 7 to 13 % in the roots. Whereas the main factor involved in the checking of vegetative development in Murneek's experiments appears to have been the nitrogen supply, large amounts of other nutrients are contained in the fruits,[2] and it seems possible that under different conditions shortage of other nutrients, e.g. carbohydrates, might lead to similar checking of vegetative development. This possibility, suggested by Murneek (1926), has received experimental support from observations on the effect of variation in carbon dioxide supply (White, 1930).

EXPERIMENTAL PROCEDURE

In order to study the effect of manurial deficiency on fruiting the method of detailed observation of the behaviour of individual blossoms, suggested by Bewley & Corbett (1930), has been developed. Observations were made throughout the season of 1933 on the fruiting of plants grown on three plots, 12 ft. sq., separated by walls of concrete. One of these plots was fertilized with complete artificials; on the other two plots nitrogen and potassium respectively had been omitted from the scheme

[1] Owen (1929) finds 39 % for the English glasshouse tomato.

[2] Murneek gives the percentage soluble carbohydrates in the fruits at ten times that in the stems. Owen (1931) finds that 41 % of the total K_2O in tomato plants is in the fruits.

of manuring over a period of years.[1] Each plot contained fifty-six plants. In order to reduce the error of the observations the marginal rows were rejected, as also were the rows adjacent to two hot-water pipes running through the plots for heating purposes. Twenty-four plants remained for observation on the no nitrogen and no potassium plots. Owing to a slightly different arrangement of the paths on the completely manured plot six more plants could be included, making thirty for this treatment. The plants were trained up trellis suspended from the roof and their growth confined to the main axis by continuous removal of incipient axillary shoots. Under these circumstances the rate of extension of the main axis, estimated from periodical measurements of height, is a useful measure of growth rate. It is essential to emphasize the distinction between rate of "growth" in height, and rate of "development" of leaves and flower-trusses from the growing point, estimated from the times at which corresponding flower-trusses unfold. It was decided to continue observations to a developmental stage corresponding to the tenth truss (blossom cluster), since the height of the plants at later stages would have necessitated the use of a ladder, which was impracticable, while fruit on higher trusses was unlikely to mature before the onset of winter. Observations were commenced on seventy-eight plants and continued as far as possible daily throughout the season with the exception of Sundays, observations for these days being estimated from the previous and subsequent days. Records of fifteen plants had to be discontinued from time to time owing to destruction by accident and insect or fungal attack. Seeds were sown of the variety Ailsa Craig on 28 December. The seedlings were given normal manurial treatment in the seed boxes and in 3 in. pots to which they were transplanted on 19 January. Planting out into the different plots took place on 3 March. The first observation was made on 24 March and the final observation on 13 October following.

Observations of the effect of nitrogen and potassium

(a) *Number of blossom-buds and fruits*

Table I (columns 2, 3 and 4) and Fig. 1 give the mean number of blossom-buds formed per truss. The third trusses have the greatest number of blossom-buds and their formation is affected by the nitrogen supply, since the mean number throughout the season is consistently low for the nitrogen-starved plants. Blossom-bud formation is not

[1] For details of the manuring see the *Cheshunt Experimental Station Annual Reports*, 1916–29.

affected by low potassium supply, and this suggests that the growing points of potassium-starved plants receive an ample nitrogen supply throughout the season.

Table I (columns 5, 6 and 7) and Fig. 2 give the mean number of fruits formed per truss. The number of fruits is greatest for the second truss of the fully manured plants and falls as the season proceeds but shows partial recovery on the eighth and ninth trusses. Variation in number of fruits of the nitrogen-starved and potassium-starved plants follows a similar course, but the mean number is reduced by both nitrogen and potassium starvation.

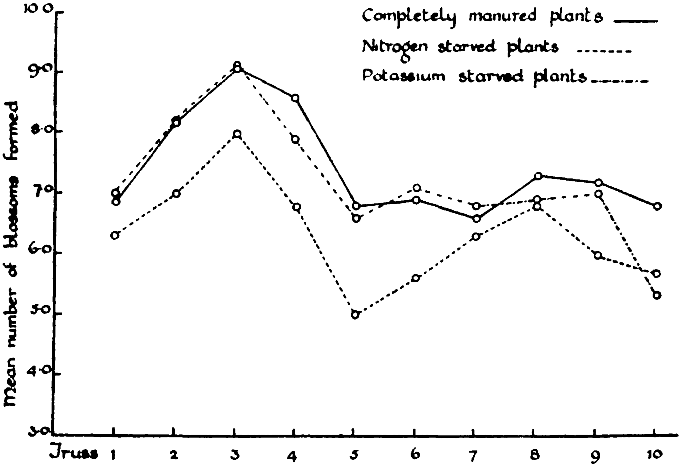

Fig. 1. Mean number of blossom-buds formed on different trusses (numbered from base to apex of the plants) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

Table I

C.A. = completely-manured plants
– N = nitrogen-starved plants
– K = potassium-starved plants

	Mean number of blossom-buds formed			Mean number of fruits per truss			% blossoms opening of buds formed		
Truss	C.A.	– N	– K	C.A.	– N	– K	C.A.	– N	– K
1	6·9	6·3	7·0	6·7	6·3	6·5	100	100	100
2	8·2	7·0	8·2	7·1	5·8	4·8	100	99	98
3	9·1	8·0	9·2	6·8	5·3	5·6	95	91	95
4	8·6	6·8	7·9	5·6	2·4	4·1	77	57	75
5	6·8	5·0	6·6	3·4	1·4	1·3	56	36	29
6	6·9	5·6	7·1	2·4	1·0	1·2	45	45	66
7	6·6	6·3	6·8	2·3	1·7	1·2	59	68	84
8	7·3	6·8	6·9	3·3	2·1	1·6	92	77	71
9	7·2	6·0	7·0	3·5	1·1	1·3	89	83	79
10	6·8	5·7	5·3	2·4	0·7	0·3	79	68	62

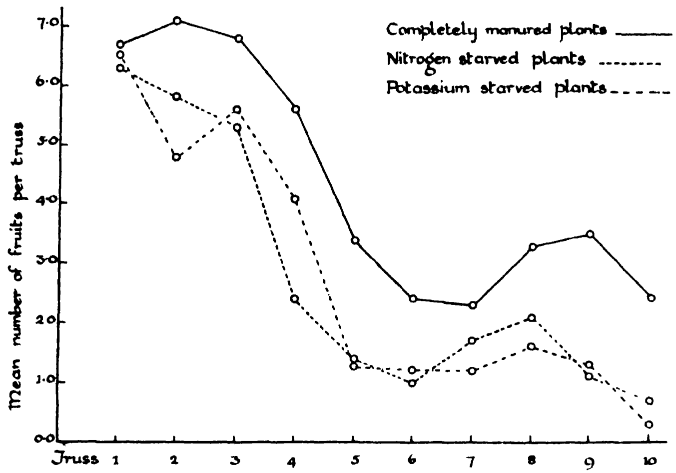

Fig. 2. Mean number of fruits borne on different trusses (numbered from base to apex of the plants) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

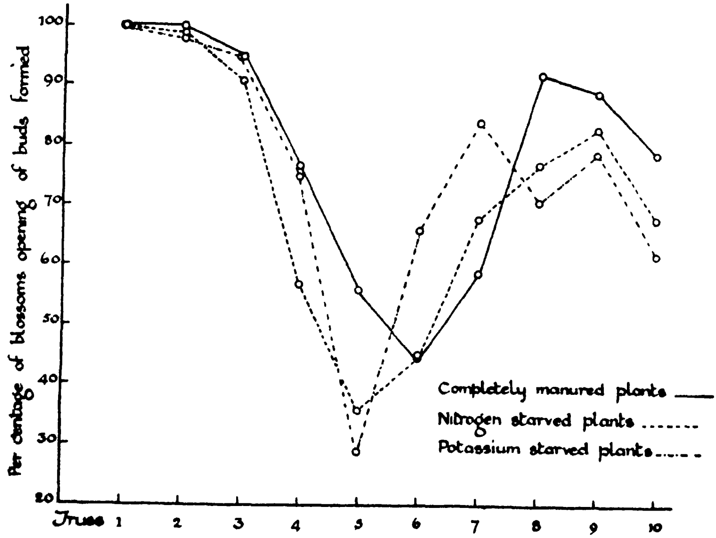

Fig. 3. Percentage blossoms opening of buds formed on different trusses (numbered from base to apex of the plants) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

Table I (columns 8, 9 and 10) and Fig. 3 give the percentage of blossoms opening of buds formed. Fig. 3 shows that for all three treatments this percentage falls sharply, reaching a minimum at the sixth truss of 45 % for the fully manured plants, and minima of 29 and 36 % at the fifth truss for the potassium-starved and nitrogen-starved plants. The high proportion of aborted buds, 55 % in the case of the fully manured plants, doubtless reflects competition for nutrient supply with the developing fruits of the second, third and fourth trusses. After maturation of these fruits a greater percentage of the buds formed on higher trusses are able to develop to the blossoming stage. On only one of the first seven trusses is the percentage of blossoms opening on potassium-starved plants markedly inferior to that of the fully-manured plants, and it appears, therefore, that the relatively low number of fruits on the potassium-starved plants (Fig. 2) is due to failure of pollination.

(*b*) *Rate of development of flower-trusses*

Records of dates of opening of the blossoms for each treatment throughout the season are too voluminous to reproduce in full: the means are given in Table II and Fig. 4. The number of blossoms opening per truss naturally varies with the individual plant, and the mean values given for apical blossoms are those where four or more replicates are available. The most satisfactory method of analysing these results would be to apply Fisher's analysis of variance (Fisher, 1935). This, however, has not been attempted as, even if only the first six blossoms of the trusses were included, the number of observations would approximate to 3 treatments $\times$ 10 trusses $\times$ 6 blossoms $\times$ 24 replicates $=4320$. The significance of the differences between trusses may, however, be estimated by comparison of the differences between corresponding blossoms of different treatments by "Student's" method of t (Fisher, 1936). The results can then be combined for each truss by taking advantage of the fact that the variance of the sum of a number of means is equal to the sum of the separate variances.

The dates of opening of the blossoms in the fully manured plants may first be considered. Since, with the partial exception of the two intervals separating the basal three trusses (which correspond with a development of five leaves, in 32 and 21 % of plants respectively), three leaves separate each truss, the relative differences in time between the opening of the basal blossoms of the trusses afford a measure of the rate of development of the growing point throughout the season (see Appendix). These differences shorten until the fourth truss is reached, lengthen between

Table II

Mean dates of opening of blossom-buds throughout the season

Columns = fruits numbered from base to apex of the trusses
Rows = trusses numbered from base to apex of the plants

	1	2	3	4	5	6	7	8	9	10
				Completely manured plants						
1	29·3	8·4	17·4	25·4	5·5	14·5	28·5	13·6	19·6	27·6
2	31·3	10·4	18·4	26·4	6·5	16·5	2·6	14·6	21·6	28·6
3	2·4	11·4	19·4	27·4	7·5	17·5	9·6	15·6	23·6	29·6
4	4·4	12·4	20·4	29·4	9·5	20·5	13·6	16·6	24·6	1·7
5	5·4	14·4	22·4	1·5	10·5	21·5	13·6	18·6	25·6	2·7
6	7·4	15·4	23·4	2·5	12·5	25·5	16·6	20·6	26·6	4·7
7	9·4	16·4	25·4	5·5	—	13·6	18·6	24·6	27·6	3·7
8	—	18·4	27·4	6·5	—	—	—	29·6	29·6	—
9	—	20·4	28·4	8·5	—	—	—	—	—	—
				Nitrogen-starved plants						
1	29·3	9·4	19·4	28·4	9·5	12·6	5·7	17·7	1·8	13·8
2	31·3	10·4	20·4	28·4	10·5	15·6	5·7	20·7	2·8	12·8
3	2·4	12·4	21·4	29·4	11·5	22·6	8·7	23·7	4·8	14·8
4	4·4	13·4	22·4	30·4	13·5	30·6	12·7	25·7	7·8	16·8
5	5·4	15·4	24·4	3·5	—	3·7	16·7	27·7	8·8	18·8
6	7·4	16·4	25·4	3·5	—	5·7	17·7	28·7	12·8	18·8
7	8·4	18·4	26·4	—	—	—	18·7	28·7	11·8	—
8	—	18·4	27·4	—	—	—	—	—	—	—
				Potassium-starved plants						
1	28·3	6·4	14·4	22·4	3·5	14·5	24·5	2·6	9·6	20·6
2	29·3	8·4	15·4	23·4	3·5	15·5	26·5	3·6	11·6	21·6
3	31·3	9·4	16·4	24·4	7·5	18·5	28·5	4·6	12·6	21·6
4	2·4	10·4	18·4	25·4	10·5	20·5	30·5	5·6	13·6	22·6
5	4·4	12·4	19·4	27·4	17·5	23·5	1·6	7·6	16·6	—
6	6·4	14·4	21·4	29·4	—	26·5	2·6	8·6	18·6	26·6
7	8·4	15·4	22·4	2·5	—	28·5	6·6	10·6	16·6	—
8	—	17·4	24·4	—	—	—	—	—	—	—
9	—	20·4	24·4	—	—	—	—	—	—	—

the fourth and seventh trusses and then shorten again until the ninth truss. Corresponding differences may be seen in the behaviour of later blossoms of each truss. The periods between the opening of adjacent blossoms in truss 1 approximate to regular 2-day intervals, and it is apparent that the slopes of the curves afford a measure of the rate of development of each truss. The steepest slope is recorded for truss 2, and, thereafter, the slopes show a progressive falling off until there are signs in truss 6 of a marked retardation of development. This process is accentuated in the early blossoms of truss 7. The mean slope of truss 8 is higher, and the intervals of truss 9 do not differ from those of truss 3 before the retardation sets in. The period of greatest retardation lies between 21 May and 13 June, corresponding to and slightly preceding (since the basal blossom of the youngest truss opens when situated a few cm. from the growing point) the opening of blossoms on the seventh and eighth trusses. This period synchronizes with the final stages of develop-

ment of the fruit on trusses 2, 3 and 4, which together constitute the major portion of the crop.

Comparison of the rates of development of completely manured plants with potassium-starved and nitrogen-starved plants in Fig. 4 reveals several striking features.

(1) The blossoms of potassium-starved plants open *earlier* than corresponding blossoms of completely manured plants. Differences of as much as 11 days between corresponding trusses are recorded in favour of the potassium-starved plants. In order to remove any doubt as to the significance of this effect, "Student's" method of t (Fisher, 1936) has been used to compare the means of corresponding blossoms of truss 3, which show differences of about 3 days. The results are shown in Table III (columns 2, 3. 4 and 5), the low values of P demonstrating the precision that may be obtained by the use of a large number of replicates. There is clear evidence of an acceleration by potassium starvation of the rate of development of successive flower trusses from the growing point.

Table III

Comparison of differences in dates of opening of blossom-buds (numbered from base to apex) of third truss by "Student's" method of "t"

	Completely manured and potassium-starved plants				Completely manured and nitrogen-starved plants			
Fruit no.	Mean difference in days	n_1+n_2	t	P	Mean difference in days	n_1+n_2	t	P
1	3·0	43	3·51	<0·01	2·0	46	2·32	0·02
2	3·0	43	3·51	<0·01	2·0	46	2·24	0·03
3	3·0	43	3·39	<0·01	2·0	46	2·20	0·03
4	2·0	43	2·36	0·03	2·0	45	2·10	0·04
5	3·0	43	3·03	<0·01	2·0	45	1·90	0·06
6	2·0	43	1·97	0·05	2·0	44	1·92	0·06
7	3·0	43	2·89	<0·01	1·0	41	0·87	—
8	3·0	40	2·80	<0·01	±0·0	35	—	—
9	4·0	26	5·28	<0·01	—	—	—	—

(2) The check to rate of development in mid-season and subsequent recovery is *accelerated* in potassium-starved plants. The trusses showing the greatest retardation are the fourth and fifth as against the sixth and seventh of the completely manured plants. Recovery is nearly complete in the seventh truss of the potassium-starved plants which takes 13 days for the opening of seven blossoms, whereas the opening of seven blossoms of the completely manured plants takes 21 days. The intervals between the opening of blossoms of the eighth truss of the potassium-starved plants do not differ from those of truss 3 before retardation sets in,

whereas the intervals between the opening of blossoms of truss 8 of the completely manured plants reveal definite signs of retarded development.

(3) The blossoms of nitrogen-starved plants, with the exception of those of the first truss, open *later* than corresponding blossoms of completely manured plants. In order to test the significance of this effect, in the early part of the season before the checking of development sets in, the means for the blossoms of truss 3 are compared in Table III (columns 6, 7, 8 and 9) with the corresponding means for the blossoms of the completely manured plants. The value of t for the whole truss, obtained from a comparison of the sums of the means, is 4·31, which corresponds with $P = <0{\cdot}01$. Nitrogen starvation and potassium starvation thus have contrasting effects on the rate of development of successive trusses from the growing point.

(4) A remarkable feature of Fig. 4 is the influence of nitrogen on the mid-seasonal checking of vegetative development, which is common to all treatments and has been associated with competition for nutrient supply with the developing fruits of trusses 2, 3 and 4. The first effect of nitrogen starvation is failure of the buds formed to open, so that the fifth truss of the typical nitrogen-starved plant consists of only four blossoms. At this stage development practically ceases, for the basal blossom of the next truss does not open until more than 4 weeks later. Thus the rate of development of the growing point must be so slow as to correspond with the unfolding of only three leaves in 4 weeks. Moreover, the sixth truss takes 23 days to open. Succeeding trusses recover slowly, but their slopes do not reach the levels of those trusses that blossom prior to the mid-seasonal check. The differences between the treatments become so pronounced that the basal blossom of the tenth truss of a typical nitrogen-starved plant opens about 2 months later than the corresponding blossom of a typical potassium-starved plant.

The marked retardation of blossoming associated with nitrogen starvation demonstrates that nitrogen is a factor of prime importance in determining the rate of development of flower-trusses from the growing point. The accentuation of the mid-seasonal check by nitrogen starvation is in agreement with the conclusions of Murneek (1926) that developing tomato fruits tend to monopolize the nitrogen supply bringing about a condition of nitrogen starvation in other parts of the plant.

(c) *Relative effect of starvation throughout the season*

The favourable development of the potassium-starved plants in comparison with the completely manured plants raises the question of to what extent were the plants on the no potassium plot really potassium-starved. Observations were made on fruit developing from the blossoms noted in Table II and Fig. 4. When mature this fruit was graded by eye into four classes corresponding to those used in the commercial marketing of tomatoes. From the mean weight of these grades the total weight of fruit corresponding with each truss for each treatment has been calculated. The weight of crop of the nitrogen-starved and potassium-starved plants, estimated as a percentage of the fruit borne by the completely manured plants, is shown in Fig. 5, which possesses several points of interest.

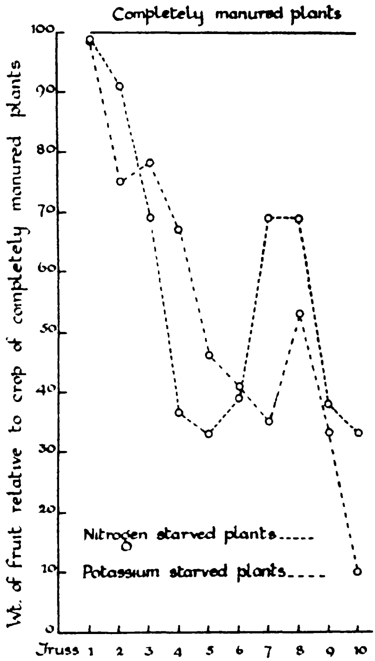

Fig. 5. Fluctuation in weight of fruit, relative to that of corresponding trusses of completely manured plants, of different trusses (numbered from base to apex of the plants) of (*a*) nitrogen-starved plants, and (*b*) potassium-starved plants.

(1) The plants on the potassium-starved plots are suffering so severely from potassium starvation that the crop on the seventh, eighth, ninth and tenth trusses is only 30 % of that of the completely manured plants. It is clear that the conditions controlling development of floral organs are very different from those subsequently necessary for the production of a heavy crop.

(2) In so far as weight of crop may be used as an indication of the degree of starvation there is no essential difference in degree of starvation between the nitrogen-starved and potassium-starved plants. The starvation effect is negligible for the first truss but increases rapidly as the season advances.

(3) There is clear indication in the case of the nitrogen-starved plants of an accentuation of the starvation effect on the fourth, fifth and sixth

trusses and a recovery on the seventh and eighth trusses. The relative effect of a low nitrogen supply is thus most marked at the period corresponding to heavy bearing of fruit. It is evident that the form of the curve showing the percentage relationship of the crop of the nitrogen-starved plants to that of the completely manured plants is that expected on the view that development of a heavy crop is controlled by the available nitrogen supply.

(*d*) *Rate of growth*

Table IV and Fig. 6 give the mean heights of the plants on each plot from 10 April until further measurements became impracticable owing to the height of the completely manured plants. Growth measurements corresponding to all treatments clearly represent two stages—a period of progressive falling off terminating in almost complete cessation of growth, followed by a period of recovery, when the relationship between height and time becomes linear. It is of interest to compare these results with the corresponding weights, shown in Table V, of mature fruit picked. The maximal weights of fruit matured for any 6 days during the season were picked between 27 May and 2 June, and this period approximates closely to that of recovery in growth rate.

Table IV

Mean height of plants each trimmed to a single axis (cm.)

Date	Completely manured	Nitrogen-starved	Potassium-starved
10 April	68	55	69
17 „	89	73	87
24 „	104	83	97
1 May	120	93	103
8 „	128	95	107
15 „	138	97	115
22 „	141	98	122
29 „	144	98	132
5 June	156	101	141
12 „	173	104	147
19 „	179	106	157

Table V

Weight of fruit in oz. maturing during successive 6-day periods

Date	Completely manured	Nitrogen-starved	Potassium-starved
6–12 May	—	8	10
13–19 „	72	111	85
20–26 „	358	384	316
27 May–2 June	478	349	353
3– 9 June	334	152	118
10–16 „	271	102	125
17–23 „	65	23	76

At a period corresponding to development of the second truss there is no difference in rate of growth between the completely manured and potassium-starved plants. Subsequently, the falling off in rate of growth of the potassium-starved plants is more severe and the slope of the curve after recovery is less steep. *Potassium starvation is thus responsible at the same time for acceleration of development* (Fig. 4) *and retardation of growth* (Fig. 6). The retardation of growth of the nitrogen-starved plants during the latter part of May is striking and emphasizes the importance of the effect of nitrogen supply on the severity of vegetative checking, that is associated with heavy bearing of fruit.

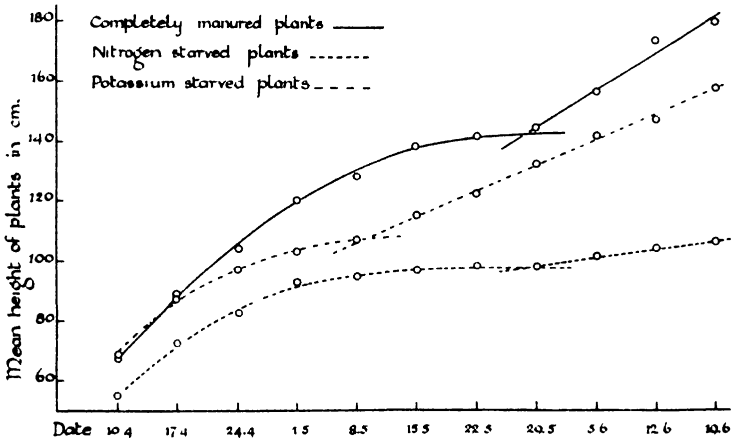

Fig. 6. Mean height in cm., at weekly intervals from 10 April to 19 June, of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

(*e*) "*Maturation*" *period of fruits*

Observations on the process of fruiting were continued by noting the dates on which each fruit became mature. Maturity was taken as corresponding to the first appearance of the orange-red pigment of the fully ripe tomato, commercial fruit being usually picked at this stage. The period between the opening of the blossom and the picking of the fruit has been termed by Bewley & Corbett (1930) the "maturation period".

As pointed out by Bewley & Corbett (1930) and White (1930) the maturation periods of fruits developing on the second, third and fourth trusses do not show regular intervals from the basal to apical fruits of a truss. The maturation periods of the basal fruits are relatively constant

and there is then a marked break of from 15 to 25 days before the apical fruits mature. White (1930) suggested that this break was due to a shortage of nutrient material, the development of the later formed fruits being held up until the basal fruits had matured.

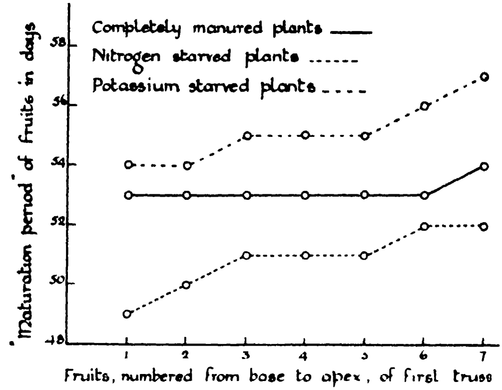

Fig. 7. Mean period between opening of the blossom and maturation of the fruit for different fruits, numbered from base to apex, of the basal truss of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

Table VI

Maturation period of fruits of completely-manured plants (C.A.), *nitrogen-starved plants* (– N) *and potassium-starved plants* (– K)

	Truss 1			Truss 2 (retarded apical fruit in brackets)			Truss 3		
Fruit	C.A.	- N	- K	C.A.	N	- K	C.A.	N	K
1	53	49	54	50	50	54	52	53	57
2	53	50	54	51	50	54	52	54	57
3	53	51	55	52	51	56	51	55	
4	53	51	55	53	51 (82)	57 (81)	52		61
5	53	51	55	54	54 (90)	- (80)	52		
6	53	52	56	55 (80)	(84)	-	53	–	
7	54	52	57	55 (87)	- (85)	—	—	—	-
8	—	—	—	(93)	–	—	-		—

Table VI (columns 2, 3 and 4) and Fig. 7 show the maturation period of fruits of the first truss. For the completely manured plants the maturation period is constant at 53 days. The mean maturation period of the basal fruits of the nitrogen-starved plants corresponds with a gain of 4 days in ripening, but this difference tends to be lost as the later-formed fruits mature. The mean maturation period of the basal fruit of the potassium-starved plants is 1 day longer than that of the completely

manured plants, and this difference is accentuated with later-formed fruit. "Student's" method of t (Fisher, 1936) has been used to test these differences and the results are shown in Table VII. The maturation period of the nitrogen-starved plants is significantly low for six of the seven blossoms. The maturation period of the potassium-starved plants, excluding the first two fruits, is significantly high.

Table VII

Comparison of differences in period of ripening of fruits (numbered from base to apex) of first truss by "Student's" method of "t"

	Completely manured and nitrogen-starved plants				Completely manured and potassium-starved plants			
Fruit no.	Mean difference in days	n_1+n_2	t	P	Mean difference in days	n_1+n_2	t	P
1	4·0	44	4·96	<0·01	1·0	41	1·47	0·14
2	3·0	43	5·19	<0·01	1·0	39	1·51	0·14
3	2·0	43	3·16	<0·01	2·0	40	2·70	0·01
4	2·0	44	3·70	<0·01	2·0	41	2·58	0·02
5	2·0	43	3·44	<0·01	2·0	39	3·46	<0·01
6	1·0	42	1·54	0·12	3·0	39	3·69	<0·01
7	2·0	25	2·76	0·01	3·0	27	3·42	<0·01

Table VIII

Mean maturation period for different trusses (excluding retarded apical fruit)

Truss	Completely manured	Nitrogen starved	Potassium starved
1	53	51	55
2	52	51	55
3	52	51	59
4	52	51	58
5	57	56	57
6	56	53	56
7	53	55	55
8	52	54	59
9	51	54	58
10	51	56	59

Table VI (columns 5, 6 and 7) and Fig. 8 give the maturation period of the second truss. The values for the completely manured fruits contrast with those for truss 1, since they lengthen regularly as the later-formed fruits mature. The retardation in ripening of the potassium-starved plants is accentuated as compared with truss 1 and the low maturation period of the nitrogen-starved plants is tending to disappear. The means of the apical "retarded" fruit are plotted separately and do not fall below 80 days for any treatment.

The mean maturation periods for the (non-retarded) fruit of each truss are given in Table VIII and Fig. 9. Potassium starvation is

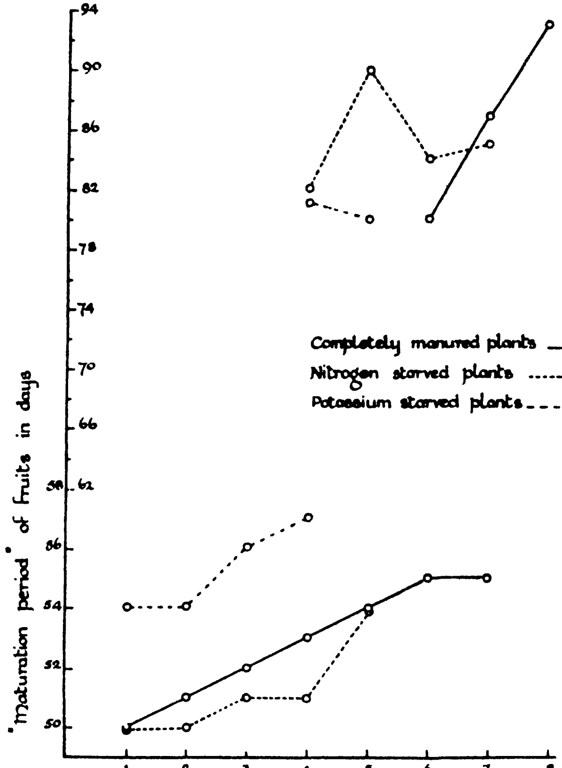

Fig. 8. Mean period between opening of the blossom and maturation of the fruit for different fruits, numbered from base to apex, of the second truss (from the base) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants. The striking difference in ripening period between the basal fruits and those apical fruits which show retarded development (plotted separately) may be noted. The values for these "retarded" apical fruits are plotted on half-scale in order to bring them on to the same diagram.

associated throughout the season with retardation in the ripening period and the acceleration in ripening of the fruit of the lower trusses of the nitrogen-starved plants is lost as the season advances. The ripening period of the completely manured plants is appreciably prolonged for

trusses 5 and 6 but subsequently shows complete recovery and is apparently independent of the age of the plant.

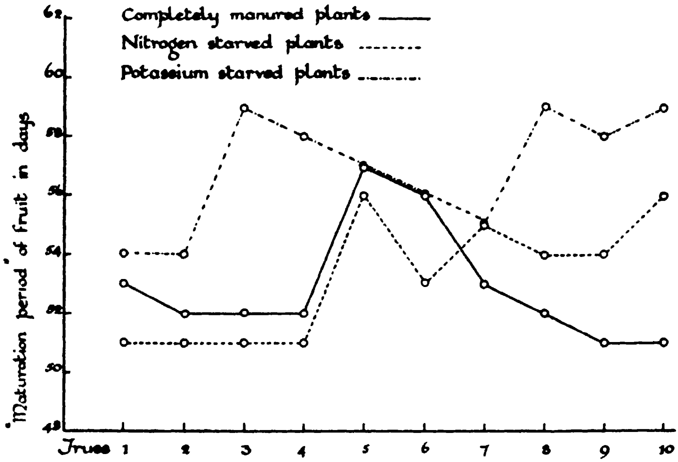

Fig. 9. Mean maturation period of fruits (excluding apical fruits with arrested development) of different trusses (numbered from base to apex of the plants) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

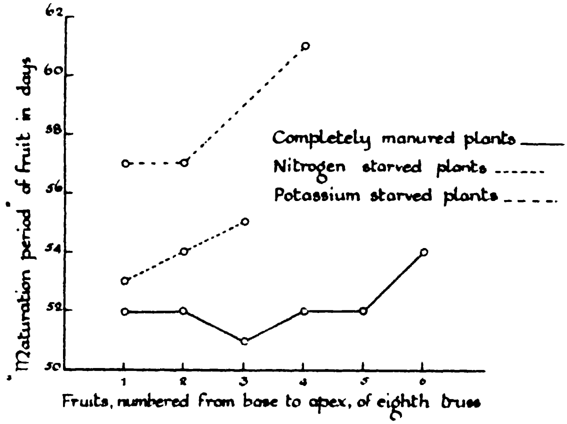

Fig. 10. Mean period between opening of the blossom and maturation of the fruit for different fruits, numbered from base to apex, of the eighth truss (from the base) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

Table VI (columns 8, 9 and 10) and Fig. 10 give the maturation period of the fruits of the eighth truss. The maturation period of different

fruits of the completely manured plants approximates to a constant as in truss 1, whereas all intermediate trusses show a tendency to lengthen for later-formed fruits, in addition to a well-marked prolongation in ripening of the apical "retarded" fruits.

Table IX and Fig. 11 give the relative number of apical fruits that were retarded in development until the basal fruits of their trusses had

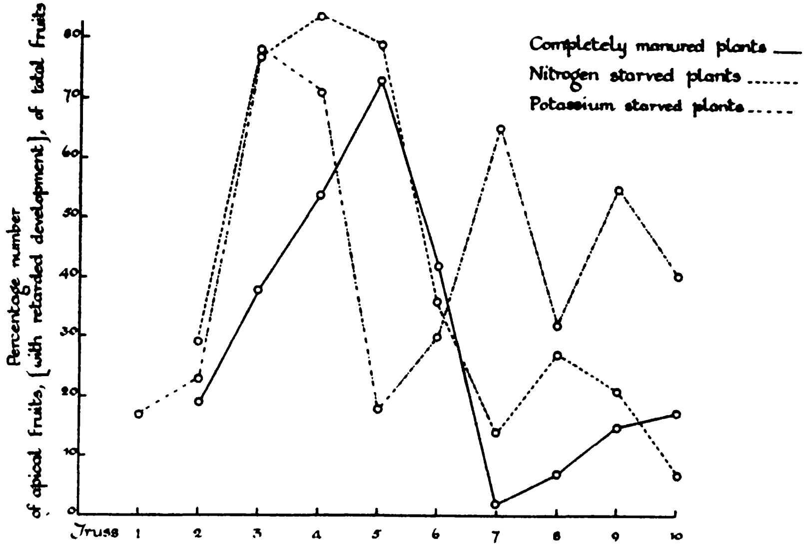

Fig. 11. Mean numbers of fruits with retarded development as percentages of the total numbers of fruits borne on different trusses (numbered from base to apex of the plants) of (*a*) completely manured plants, (*b*) nitrogen-starved plants, and (*c*) potassium-starved plants.

Table IX

Numbers of fruits of different trusses: (a) ripening normally, (b) with arrested development

Truss	Completely manured plants (*a*)	Completely manured plants (*b*)	Nitrogen-starved plants (*a*)	Nitrogen-starved plants (*b*)	Potassium-starved plants (*a*)	Potassium-starved plants (*b*)
1	166	1	132	—	114	2
2	143	34	87	35	63	19
3	106	64	26	85	21	74
4	64	76	8	43	20	49
5	23	61	6	23	18	4
6	34	25	14	8	14	6
7	56	1	30	5	7	13
8	77	6	33	12	19	9
9	74	13	19	5	10	12
10	50	10	13	1	3	2

matured. The proportion of "retarded" apical fruit is very high for all treatments on the third, fourth and fifth trusses. These are the trusses that carry a heavy crop, and the percentage of "retarded" fruits falls when this crop has matured. A high proportion of these fruits occurs earlier in the nitrogen-starved plants in comparison with the completely manured plants, while the rise and subsequent fall is to a higher level than that of completely manured plants. It is of interest to note that the maximal percentage of "retarded" apical fruits is shifted towards the lower trusses in the potassium-starved plants. Fig. 11 shows that the proportion of "retarded" apical fruit is less than 20% for the fifth truss of the potassium-starved plants, whereas it is still over 70% for the corresponding truss of the completely manured plants. The production of "retarded" apical fruit is accentuated by both low nitrogen supply and low potassium supply.

(*f*) *Leaf area*

Since it was impracticable to use whole leaves the area of corresponding leaflets was estimated by enclosing leaves between thin panes

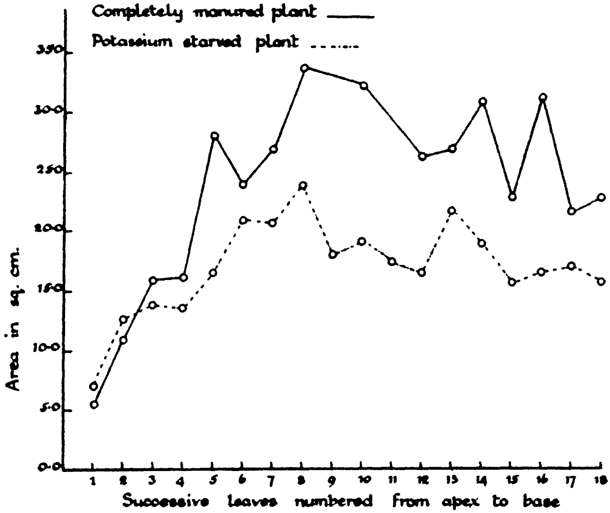

Fig. 12. Mean area in sq. cm. of corresponding leaflets of successive leaves, numbered from apex to base, of (*a*) a completely manured plant, and (*b*) a potassium-starved plant.

of glass, drawing an illuminated outline on tracing paper and tracing the areas with a planimeter. Owing to pressure of other work and the large number of estimations that would have been required, the work had to

be limited to comparison of a single plant from each treatment. Care was taken to select a normal completely manured plant and plants with characteristic though moderate symptoms of nitrogen and potassium starvation. The terminal pair of leaflets of every leaf on the plants was used.

Table X

Area (sq. cm.) of corresponding leaflets from every leaf (numbered from apex to base) of a typical plant from each treatment

Leaf no.	Completely manured	Nitrogen-starved	Potassium-starved
1	5·67 5·58 5·48	6·25 5·83 5·41	5·90 6·96 7·03
2	11·60 10·8 9·99	12·7 12·7 12·8	11·7 12·6 13·5
3	15·7 15·8 15·9	15·4 15·4 15·5	14·6 13·8 12·9
4	15·0 16·2 17·5	19·5 18·7 17·9	12·6 13·6 14·6
5	27·0 28·0 29·0	18·6 18·8 19·1	16·0 16·6 17·3
6	24·2 23·9 23·6	13·4 13·5 13·7	21·8 20·9 19·9
7	26·4 26·9 27·4	20·1 18·8 17·5	21·6 20·7 19·8
8	34·4 33·7 33·0	18·6 18·7 18·9	21·6 23·9 26·2
9	31·5 32·3 33·1	24·1 24·1 19·0	19·1 18·1 17·1
10	26·0 26·3 26·6	23·0 22·5 22·0	21·4 19·2 16·9
11	26·6 27·0 27·5	16·1 16·1 —	18·3 17·4 16·4
12	30·9 31·0 31·2	19·1 17·9 16·6	16·0 16·6 17·2
13	23·4 23·0 22·6	15·5 15·8 16·2	21·8 21·8 21·7
14	28·8 31·3 33·8	—	16·6 19·0 21·0
15	21·1 21·7 22·3	—	17·9 15·7 13·5
16	22·9 22·9 23·0	—	17·4 16·6 15·7
17	—	—	16·2 17·1 18·1
18	—	—	14·8 15·7 16·6

The results are given in Table X, which shows that the leaflets corresponding to the eighth leaf from the growing point of the completely manured plant have the greatest area and are presumably the youngest to have reached their full size. Fig. 12 shows that the greatest difference in area between the fully manured and potassium-starved plants occurs at the eighth leaf from the growing point. The decline in area in older leaflets shows that up to the period of development of the fifth truss,

when these estimates were made, successive leaves attained a progressively larger size on reaching maturity. The area of *fully matured* leaves of the potassium-starved plant continues therefore to fall off relatively to the completely manured plant as the season advances. On the other hand, the *immature* leaves of the potassium-starved plant show with decreasing age progressively less difference in area relative to the completely manured plant.

DISCUSSION OF THE EFFECT OF NITROGEN AND POTASSIUM ON FRUITING

Effect of nitrogen supply

The observations of the preceding sections are summarized below:

(1) The rate of development of flower-trusses from the growing point and the rate of increase in height of plants trimmed to a single axis are both related to the nitrogen supply, becoming slower as the season advances and the effect of nitrogen starvation becomes more acute (Tables II, IV and Figs. 4, 6).

(2) The number of blossom-buds formed, the proportion of blossom-buds formed that open, and the number of buds formed that develop into fruit are related to the nitrogen supply, being in general low in nitrogen-starved plants (Table I and Figs. 1–3).

(3) The "maturation period" (between the opening of the blossom and ripening of the fruit) is shortened by slight nitrogen deficiency but lengthened by severe nitrogen starvation (Tables VI–VIII and Figs. 7, 9).

(4) The effect of low nitrogen supply on growth and on weight of crop is particularly marked at the mid-seasonal period of heavy fruit-bearing (Table V and Figs. 5, 6).

(5) The proportion of apical fruit that are severely retarded in ripening is increased by low nitrogen supply (Table IX and Fig. 11).

(6) Leaf area is markedly reduced by low nitrogen supply (Table XIV).

These observations lead to the following conclusions:

(1) Low nitrogen supply is associated with retardation of growth and development. Every characteristic of fruiting considered is retarded by low nitrogen supply, save the period of ripening of the fruit, and this is accelerated only in the lowest trusses. Increasing severity of nitrogen starvation later in the season is associated with prolongation of the ripening period.

The writer showed in a previous publication (White, 1930) that enrichment of the atmosphere of the glasshouse with carbon dioxide led

to a shortening of the period of ripening of the lowest trusses. Thus the period of ripening of the earliest formed trusses may be shortened *either by increasing the carbon dioxide supply in the atmosphere or by reducing the nitrogen supply in the soil.* Moreover, Porter (1937) has recently shown that a similar result is associated with increase of light intensity. This effect of a high carbohydrate-nitrogen balance accords with the view that the process of ripening is accelerated by a high level of sugars passing to the fruit. In a "vegetative" plant a higher proportion of the sugars synthesized in the leaves would tend to be used in vegetative growth and a lower proportion translocated to the fruit while the converse tendency would hold for a "fruitful" plant. The prolongation of the ripening period in the potassium-deficient plants thus suggests that, in these plants, there is impaired translocation of sugars to the fruits.

(2) The check to growth and development associated with heavy fruit-bearing on the second, third and fourth trusses is related to the nitrogen supply. In nitrogen-starved plants cessation of growth is more severe, reduction in crop of the fifth and sixth trusses is accentuated, and there is a high proportion of "retarded" apical fruits on the second, third and fourth trusses. These observations are compatible with the analyses by Owen (1929, 1931) of foliage of plants grown on these experimental plots in previous years, for leaves taken near the level of the fifth truss in June, i.e. at a period of heavy fruiting (Owen, 1929, Table I, 1924–5–6) have a lower nitrogen level than leaves taken in August, i.e. at a period of relatively light fruiting (Owen, 1929, Table I, 1927). Further the nitrogen level throughout the growing season falls to a minimum in the latter half of May with subsequent recovery (Owen, 1931, Table III). A previous publication (White, 1930) showed that a high level of carbon dioxide in the atmosphere of the glasshouse tended to reduce the severity of the mid-seasonal check, which seems therefore to be due to a general low level of nutrition. It appears that fruiting proceeds unregulated by the capacity of the plant to bear the number of blossoms opened and fruit set. The second, third and fourth trusses unfold at a period of the season when rates of growth and development are rapid and the intervals between the opening of blossoms are relatively short. This leads to the setting of a large number of fruits in a relatively short period. The demand for sugars and protein by the developing fruits increases until it exceeds the supply which is sufficient, even with temporary cessation of development of further trusses, for only a portion of the fruit set. A characteristic feature of fruiting in the

tomato now becomes apparent. The basal fruits of the trusses become possessed of the capacity to monopolize the nutrient supply, vegetative extension practically ceasing, differentiation of leaves and flower-trusses from the growing point being brought to a standstill and the apical fruits remaining in a state of suspended development. Further vegetative activity is apparent only when the crop is reaching maturity. A second period of relatively heavy fruit-bearing, associated with similar but less intense checking of vegetative growth and development, follows later in the season.

Regulation of fruiting is practised in the commercial culture of certain crops. Thus it is well known that in the apple the problem of biennial cropping, associated with a cycle of exhaustion and recovery, may be overcome by "thinning". Theré appears to be no reason why cyclic cropping of the tomato, due to alternating periods of exhaustion and recovery, should not be corrected by similar methods. It seems probable that, if the number of fruits on the lower trusses were restricted to those likely to reach good size and quality, an earlier and more even pick would be obtained and also, ultimately, a larger marketable crop, since the elimination of a large number of small fruits that do not reach marketable size would result in conservation of nutrient supply and less complete cessation of growth in mid-season.

Effect of potassium supply

The observations of the preceding sections are summarized below:

(1) The rate of development of flower-trusses from the growing point is related to the potassium supply, being consistently accelerated in potassium-starved plants (Table II and Fig. 4).

(2) The number of blossom-buds formed is not affected by the potassium supply, fluctuations in bud formation throughout the season in potassium-starved plants being almost identical with those of completely manured plants (Table I and Fig. 1).

(3) The percentage of blossoms formed that open is not appreciably affected by low potassium supply for the first seven flower-trusses, but the percentage of blossoms opening that fail to develop into fruits is consistently reduced in potassium-starved plants, apparently through failure of pollination (Table I and Figs. 2, 3).

(4) Fruit bearing is greatly affected by potassium supply, the weight of crop being markedly and progressively reduced throughout the season in potassium-starved plants (Fig. 5).

(5) The rate of growth of potassium-starved plants, estimated from increase in height, is not appreciably affected at a stage of development corresponding to the unfolding of the second flower-truss but subsequently shows a decline in relation to the completely manured plants (Table IV and Fig. 6).

(6) The "maturation period" of the fruit is prolonged in potassium-starved plants (Tables VI–VIII and Figs. 7–10).

(7) A high proportion of fruit in a stage of arrested development occurs earlier and on lower trusses in potassium-starved plants than in completely manured plants (Table IX and Fig. 11).

(8) The onset of and recovery from the check to vegetative growth, associated with heavy fruit-bearing on the second, third and fourth trusses, is accelerated by low potassium supply (Tables II, IV and Figs. 4, 6).

(9) Leaf area is related to the potassium supply. The maximal effect of low potassium supply is shown by the youngest mature leaf, older leaves, presumably associated with lesser degrees of severity of potassium starvation, being progressively less affected. The effect of low potassium supply on reduction in leaf area also becomes progressively less with decreasing age of leaf (Table X and Fig. 12).

An outstanding feature of these observations is the acceleration of differentiation of leaves and flower-trusses from the growing point by low potassium supply. Subsequent growth, estimated from the area of mature leaves and the length of the ripening period of the fruit, is retarded. Although development of the growing point of the main axis, estimated from the times of opening of blossom-buds of successive trusses is accelerated, growth, estimated from height, is retarded. These processes of acceleration of development and retardation of growth in height are clearly shown (Figs. 4, 6) to be taking place *simultaneously*.

The dependence of the rate of development of flower-trusses from the growing point upon the nitrogen level is shown by the striking retardation of development of the nitrogen-starved plants (Fig. 4). Even with moderate nitrogen starvation there is a significant retardation of development apparent as early in the season as the opening of the blossoms of the third flower-truss (Table III) although carbohydrate level must have been high at this period, as reflected in the shortening of the ripening period of the first flower-truss (Tables III, VI and VII). It would appear that a rate of development in advance of that of completely manured plants, as with potassium starvation, must be associated with high protein level at the growing point.

These results are attributed to the following effects:

(1) Potassium is continually translocated from the older portions of the plant to the growing point. This assumption may be made with some degree of confidence for it is generally accepted to be a characteristic feature of potassium starvation in plants. Nightingale *et al.* (1928) find by microchemical tests that nearly all the potassium in tomato plants starved of this element, apart from that contained in the fruit, is localized in the stem tip and in the younger leaves. Similar results are recorded by Janssen & Bartholomew (1929). The potassium in starved plants is apparently undergoing a continuous cycle, being translocated from older parts of the plant to the growing point, incorporated in young organs and subsequently retranslocated as these organs are approaching maturity.

(2) The level of potassium supply regulates nitrogen metabolism, a moderately low potassium supply being associated with high protein level and a greater degree of severity of potassium starvation with low protein level. By plunging leaves into hot water, decolorizing in warm alcohol and staining with Millon's reagent it was confirmed, from the intensity of the stain, that older potassium-starved leaves had a low protein level while the protein level of the young leaves was found to be at least as high as that of the young leaves of the fully manured plants. Similar conclusions have been reached by Richards & Templeman (1936), who have carried out detailed chemical analyses of potassium-starved barley leaves.

The combination of these two effects readily accounts for the results. Continual translocation of potassium from the older leaves to the young tissues maintains the growing point in the high protein phase. Consequently leaves and flower trusses of the potassium-starved plants are differentiated earlier than those of the completely manured plants, and the immature leaves show no reduction in area. In contrast mature leaves and older parts of the stems, from which the potassium is being translocated, have a lower protein level than the corresponding parts of completely manured plants. Growth in height involves the extension of the stem for some distance below the growing point, as indicated by the progressive lengthening of the internodes between successive leaves. Consequently, although the development of the growing point itself is accelerated in potassium-starved plants, the height of the plants is simultaneously reduced.

Reference may be made to the characteristic features of the foliage symptoms of potassium-starved plants in these experiments. In the early part of the season moderate potassium starvation is associated

with the production of leaves of a deep green colour presumably signifying high protein content, since the depth of colour and protein content of leaves vary together, as shown by Gassner & Goeze (1934), Michael (1935) and others. Owing to translocation to the growing point the concentration of potassium is subsequently reduced below that requisite for the maintenance of a high protein level, and this effect is first apparent as a marginal chlorosis indicative of protein breakdown.[1] As translocation of potassium proceeds chlorosis extends inwards, as described by Bewley & White (1926), associated with marginal drying out of the tissues or "scorch", until, finally, only the main veins retain their deep green colour. As the quantity of fruit hanging on the plant increases, the nitrogen level becomes insufficient to permit of both vegetative growth and fruit development and the concentration of protein at the growing point falls, associated with retardation of vegetative growth. At this stage very small leaves are produced of a pale yellow colour which resemble closely the leaves of plants on the nitrogen-starved plot. Later in the season, when the weight of fruit hanging on the plants is less heavy, larger leaves of a deep green colour are again produced.

These effects are in agreement with the results of other investigations of the influence of potassium starvation on the tomato. The type of growth obtained in the Cheshunt experiments resembles that noted by Nightingale *et al.* (1930) in that, in both cases, fruiting is associated with a nitrogen-starved phase, followed by the production of shoots with deep green leaves. No chemical analyses were made by Nightingale *et al.* on foliage collected during this nitrogen-starved phase, but those of Owen (1931, Table I) indicate, in each of 3 years, a low protein level of the leaves. On the other hand, it is noteworthy that in the investigations of Janssen & Bartholomew (1929), in which at the end of the experiment "very few fruits had set", and in those of Phillips *et al.* (1934), who removed all blossom-clusters as they were formed, there is no mention of a nitrogen-starved phase, while chemical analyses show a high protein level of the leaves.[2]

[1] Attention has been directed to the association of low potassium supply with protein breakdown by Richards & Templeman (1936).

[2] Sheng-Han Shih (London Univ. Ph.D. Thesis, 1936) has demonstrated that the type of growth associated with potassium starvation in cereals is affected by the level of sodium manuring. The effect of potassium deficiency on cereals in relation to the level of other nutrients, including sodium, is discussed by Gregory in a recent review (*Ann. Rev. Biochem.* Stanford Univ. 1937, pp. 557–78). It is of interest to note that potassium was replaced by sodium by Nightingale *et al.* and Phillips *et al.* but not by Janssen & Bartholomew or in the Cheshunt experiments.

The blossoms of the third flower-truss of the potassium-starved plants open at a significantly earlier date than the corresponding blossoms of the completely manured plants (Table III), although at the same period there is no difference in height (Table IV). *The acceleration of development associated with potassium starvation thus precedes reduction in growth rate.* This suggests that the high protein level to which this acceleration of development has been attributed must be a relatively direct effect of moderate potassium starvation on protein synthesis and not due merely to nitrogen accumulation associated with a reduced size of plant and unchanged rate of nitrate absorption.

The first sign of limitation of fruiting by potassium deficiency in the present experiments was failure of pollination (p. 25). Howlett (1936) records that pollen sterility in the tomato is associated with carbohydrate deficiency in contrast to nitrogen deficiency, which does not have this effect. As in the case of prolongation of the maturation period (p. 40) the effect of potassium deficiency is similar to that of carbohydrate deficiency relative to nitrogen supply. These two effects suggest that correction of the carbohydrate-nitrogen balance is an important result of potassium manuring, a view supported by the association of dark green leaves, indicative of high protein level, with the early stages of potassium deficiency, and by the acceleration of development of flower trusses from the growing point.

Owing to the deep colour and healthy appearance of the foliage of tomato plants in the early stages of potassium deficiency in association with relatively rapid vegetative development the necessity for potassium manuring to maintain fruit production is apt to be overlooked. A plant subject to the early stages of potassium deficiency under the conditions of the present experiment is relatively tall with deep green leaves, and characterized by accelerated blossoming but failure of pollination. At a more severe stage of starvation the marginal chlorosis developing into scorch in association with blotchy ripening of the fruit are distinctive diagnostic features (Bewley & White, 1926).

SUMMARY

1. The effect of nitrogen and potassium on the fruiting of the tomato under glass is studied by observations on plants grown on completely manured plots and plots from which nitrogen and potassium respectively have been omitted from the scheme of manuring over a period of years.

2. Nitrogen starvation reduces the number of blossom-buds formed,

the percentage of buds that open and the mean numbers of fruits per flower-truss. Potassium starvation does not affect the number of blossom-buds formed or the percentage of buds that open but reduces the mean numbers of fruits per flower-truss.

3. An outstanding feature of nitrogen starvation is retardation of the rate of development of successive flower-trusses. With potassium starvation the rate of development is accelerated. Growth, estimated from increase in height of plants trimmed to a single axis by continuous removal of incipient axillary shoots, is retarded by both nitrogen and potassium starvation.

4. The "maturation period" (between opening of the blossom and ripening of the fruit) is lengthened by potassium starvation, shortened by moderate nitrogen starvation and lengthened by severe nitrogen starvation. From the present results, in conjunction with data published previously, it is concluded that the "maturation period" is shortened by a high carbohydrate-nitrogen balance and lengthened by a low carbohydrate-nitrogen balance.

5. In mid-season all plants suffer a check to growth and development demonstrated by (*a*) retardation of the rate of differentiation of leaves and flower-trusses, (*b*) cessation of growth in height, and (*c*) the occurrence of a high proportion of fruit with arrested development. This "mid-seasonal check" is accentuated by nitrogen and potassium starvation, especially the former, corresponds with the period of maximal weight of developing fruit, and is attributed to competition for nutrient supply between the fruits and vegetative parts of the plants, leading to apparent antagonism between these processes.

6. The fruiting and foliage symptoms of the potassium-starved plants are attributed to the following effects:

(*a*) The potassium in starved plants is undergoing a continuous cycle, being translocated from older parts of the plant to the growing point, incorporated in young leaves and flower trusses and subsequently re-translocated as these organs are approaching maturity.

(*b*) The level of potassium supply regulates nitrogen metabolism, a moderately low potassium supply being associated with high protein level and a greater degree of severity of potassium starvation with low protein level.

7. The results are discussed in relation to the problems of over-bearing and potassium manuring. The observed effects of potassium deficiency on fruiting (acceleration of development of flower trusses, failure of pollination and prolongation of the "maturation period" of

the fruit) are those also associated with carbohydrate deficiency relative to nitrogen supply.

In conclusion the author is indebted to Dr W. F. Bewley for permission to publish this paper and to Mr A. D. Goddard for assistance in carrying out the observations.

APPENDIX

The effect of nitrogen and potassium on order of development of leaves and flower-trusses

In the present experiments the times of opening of corresponding buds of corresponding flower-trusses have been used to estimate rates of development. Since the use of such a measure is invalidated if the treatments considered suppress the differentiation of flower-trusses or affect the number of leaves separating corresponding trusses, it is essential to consider the effect of manurial deficiency on the *order* of development of flower trusses and leaves.

In order to study the effect of manurial treatment on the position of the first-formed truss an experiment was carried out in which seed of the variety E.S.I. was divided into two batches. One batch was grown in soil known to be nitrogen-deficient. The pale yellow seedlings had stiff stems with small leaves and were clearly nitrogen-starved. The other batch was grown in the same soil enriched with a dressing of dried blood and watered periodically with nitrate. These seedlings had a high nitrogen content, as indicated by their deep green colour, succulent foliage and tendency for rapid development of incipient axillary shoots. Nevertheless, the difference in nitrogen level did not affect significantly the mean number of leaves between the cotyledons and the first truss, which was 9·5 for the nitrogen-deficient plants and 9·3 for the plants with ample nitrogen supply. Moreover, since in the present experiments the basal blossom of the first-formed truss opened within 21 days of planting, it is probable that the position of this truss had been determined before the effects of manurial deficiency became operative.

Three leaves invariably separated the flower-trusses of the main axis in the present experiment with the exception of the intervals between the first and second and second and third trusses, which consisted of five leaves in a variable percentage of plants. This percentage differs from season to season but these differences, shown in Table XI, cannot be related to variation in manurial treatment. The largest difference in

comparison with the completely manured plants is shown by the nitrogen-starved plants for the leaf interval between the second and third trusses, for 15 % more of these plants possess five or more leaves instead of the three or four leaves characteristic of the completely manured plants. This difference is not sufficiently consistent to be considered significant ($P = 0{\cdot}15$). The assumption may, therefore, justifiably be made that differences in times of opening of corresponding buds of different trusses are due to the effects of manurial treatment on the rate of development of the growing point.

Table XI

Relative effect of manurial deficiency on number of leaves developing between flower-trusses

(The percentages of plants with an even number of leaves are mainly due to abnormality such as the production of opposite leaves)

Between first and second flower-trusses

	3				4			
	1933	1935	1936	1937	1933	1935	1936	1937
Completely manured	61	33	43	53	7	8	10	17
Nitrogen-starved	82	25	67	79	—	4	—	4
Potassium-starved	92	25	50	46	—	4	—	8

	5				6			
	1933	1935	1936	1937	1933	1935	1936	1937
Completely manured	32	59	43	30	—	—	4	—
Nitrogen-starved	14	59	29	13	4	12	4	4
Potassium-starved	8	71	38	42	—	—	12	4

Between second and third flower-trusses

	0	3				4			
	1937	1933	1935	1936	1937	1933	1935	1936	1937
Completely manured	—	65	92	37	60	14	4	3	10
Nitrogen-starved	4	82	88	29	46	—	—	—	4
Potassium-starved	—	66	96	46	46	17	4	—	8

	5				6				7			
	1933	1935	1936	1937	1933	1935	1936	1937	1933	1935	1936	1937
Completely manured	21	4	53	30	—	—	7	—	—	—	—	—
Nitrogen-starved	18	12	50	46	—	—	17	—	—	—	4	—
Potassium-starved	17	—	37	46	—	—	17	—	—	—	—	—

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THE EFFECT OF MANURING UPON APPLE FRUITS

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(With 2 Text-figures)

CONTENTS

I. INTRODUCTION

SINCE 1923 one of us has investigated the control of apple scab (*Venturia inaequalis* Aderh.) in Northern Ireland orchards (Northern Ireland, 1935; Muskett & Turner, 1929) by the adoption of routine summer spraying. During the course of this work the carrying out of the same summer-spraying programme (Northern Ireland, 1927) gave consistently better results in some orchards than in others. In one particular orchard, where the measure of control obtained was usually low, it was decided to investigate whether this could be explained in any way in terms of tree nutrition and also to make observations upon the effect of manuring upon the fruit. In some ways this orchard which was situated at Dunadry, Co. Antrim, was ideal for the work. It was a well-kept grass orchard and comprised 151 trees, all of which, with one exception (Lane's Prince Albert), were of the variety Bramley's Seedling. The trees were all of the same age (20 years old in 1929), had been worked on the same stock and showed little variation in size. They were widely spaced, having been planted 20 ft. apart, and the general conditions were favourable for the carrying out of a manurial experiment. On the other hand, the orchard was in an exposed position and very susceptible to blossom damage by spring frosts. It was inclined to crop erratically and could not be regarded as entirely satisfactory from this point of view. Owing to repeated damage to the blossom by spring frosts satisfactory results from this investigation over a period of years were not obtained but, so striking and clear-cut were the results obtained in the one year (1930) in which the orchard cropped well, that it has been decided to put them on record.

II. SOIL ANALYSIS

An analysis of the soil of the Dunadry orchard was made by Mr J. C. Baird, of the Department of Agricultural Chemistry, Queen's University of Belfast. The results are given in Table I. From these data it will be seen that the soil is very deficient in nitrogen, but that the supplies of potash

Table I

Analysis of soil from Dunadry orchard

Chemical analysis		Mechanical analysis	
Moisture	6·52	Coarse sand	16·70
Nitrogen	0·231	Fine sand	24·50
Phosphoric acid (total)	0·114	Silt	21·65
Potash (total)	0·411	Clay	24·70
Phosphoric acid (available)	0·043	Loss on ignition (organic matter)	11·28
Potash (available)	0·024	Carbonates	0·044

and phosphates, as regards both total content and available material, are ample. The ground flora of the orchard for some seasons past had indicated the soil to be deficient in nitrogen.

III. Spraying programme

During the period under review the normal practice in orchards in Ulster where trees of the variety Bramley's Seedling were grown and which were not subject to attack by apple capsids was to spray the trees with a tar distillate wash in late winter for the control of apple sucker and aphides, and to follow this up with at least three summer sprayings with Bordeaux mixture for the control of apple scab (Northern Ireland,

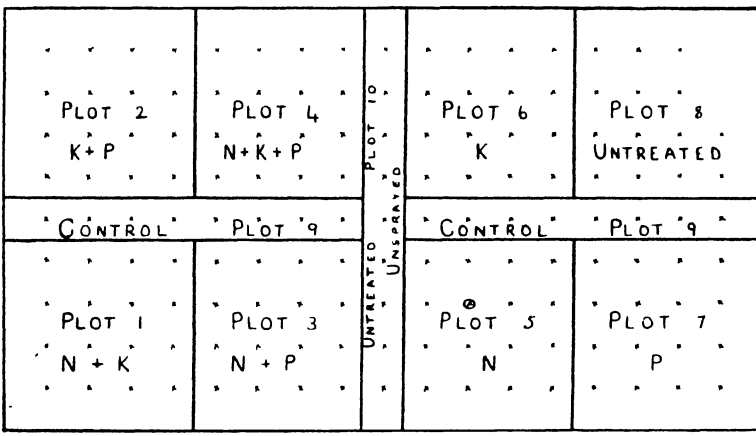

Fig. 1. The lay-out. 1929–30. × position of tree of variety Bramley's Seedling; ⊗ position of tree of variety Lane's Prince Albert.

1927). During the course of this investigation the trees, with the exception of those in plot 10 (see Fig. 1), were so treated, the only alteration being that, in 1929, no application of a tar distillate was given. The trees in plot 10, which had been used as controls during previous spraying experiments, were neither sprayed nor manured.

IV. The lay-out

In 1929 the orchard was divided into ten plots as shown in Fig. 1. Each plot with the exception of Nos. 8 and 10 contained sixteen trees of the variety Bramley's Seedling. The odd tree of the variety Lane's Prince Albert was situated in plot 5. All the plots with the exception of Nos. 8, 9 and 10 received manurial treatment. Of the unmanured

plots No. 9 represents the true control, since the trees here received the same spraying programme and similar cultural treatment to those in the manured plots. Plot 8 had been utilized for the growing of potatoes before the commencement of the experiment. Guard rows were not provided between trees in plots receiving different treatments, because of the wide planting system adopted. This lay-out was changed in 1931.

V. MANURIAL TREATMENT

The effects of manuring the trees with nitrogen, potash and phosphate, applied singly or in combination, were investigated, and the following fertilizers were employed:

Sulphate of ammonia	(20·6 % N)
Muriate (chloride) of potash	(50·0 % K_2O)
Superphosphate	(16·03 % soluble P_2O_5)

It was decided that more pronounced results would be likely to ensue from the application of larger quantities of fertilizers than those generally used and, therefore, the manures were applied as follows: sulphate of ammonia, 10 lb. per tree; muriate of potash, 5 lb. per tree; and superphosphate, 10 lb. per tree. In 1931 and 1932 certain plots received double the usual dressing of muriate of potash or superphosphate. Farmyard manure was applied in 1931 and 1932 to one plot at the rate of 10 cwt. per tree). The fertilizers were broadcast evenly around each tree and extending beyond the spread of the branches. Care was taken that none was applied within a yard of the trunk. The potash and phosphate were applied during the middle of March and the nitrogen towards the end of May.

VI. EXPERIMENTAL METHODS

At the time of picking the fruit the produce of each tree was divided into two classes, (i) apples which showed any signs of established infection by scab, and (ii) those which showed no signs of such infection. Each class was weighed separately and the weights recorded for each tree. From these figures the percentage weight of scabbed fruit borne by each tree was calculated.

The resistance of the fruit to invasion by *Cytosporina ludibunda* strain CE was determined by the method of Gregory & Horne (1928) on samples of apples selected at random in the orchard from the produce of each plot and despatched to the Imperial College, London, where the tests were carried out. Apples were inoculated on one side with the fungus and stored at laboratory temperatures until the rotted tissue was

estimated, and the radial advance of the fungus, in mm. per day, for each individual apple was determined. From these data the average mean radial advance per day was calculated for each sample.

The nitrogen content of the fruit was determined, using samples selected at random from each plot, by the Kjeldahl method according to the technique described by Archbold (1925).

The determination of the radial spread of *C. ludibunda* CE in sterilized apple pulp was made by preparing pulp from each of the various samples. The pulp was sterilized by steaming and used as a medium without the addition of any other ingredient. Plates were prepared in triplicate, and the pulp from apples of each sample inoculated with the fungus, incubated at a constant temperature and the growth of the fungus measured after 11 days.

VII. Results for 1929

During the summer of 1929 it was possible to pick out all the trees which had received a dressing of sulphate of ammonia alone or in combination with other fertilizers, since the trees receiving nitrogenous manure bore large dark green-coloured leaves. Manuring with potash or phosphate was observed to have no effect on the foliage colour. The crop in 1929 was very poor, owing to blossom damage by spring frosts, and although some colour differences were noted in the fruit no further observations were made for this year.

VIII. Results for 1930

Field observations

Apart from the darker foliage difference, it was clearly shown in 1930 that nitrogenous manuring increased the amount of blossom produced by the trees, those in the plot receiving complete manuring being adjudged the best. Furthermore, the trees receiving nitrogenous dressings were in bloom about 10 days earlier than those which were untreated or received no nitrogen. This precocious and profuse blossoming was not, however, reflected in the fruit yields (see Table II).

The trees in the plots receiving nitrogen alone or in combination with other fertilizers showed increased growth in 1930.

The crop was good for the year, and the fruit from the various plots showed striking differences in colour and texture. The fruit from the trees which had received nitrogen, alone or in combination with other fertilizers; was very green in colour and soft in texture, while that from the control, the potash and the phosphate-treated plots was highly

coloured and very hard. Little difference could be seen between plots manured with potash and with phosphate, but phosphates appeared to produce a pleasant yellowness of the skin of the fruit.

Table II shows the mean values for total yield and percentage weight of scabbed fruit from each plot. These values are each based on records of fifteen trees. The mean value for yield per tree for the nine trees in plot 10 which was untreated and unsprayed was 3·3 lb., two of the trees bearing no crop. The mean percentage weight of scabbed fruit borne by each tree which cropped in plot 10 was 61·5.

Observations on resistance to fungal invasion

A sample of forty apples from each of nine plots was available for the determination of resistance to fungal invasion by the method of radial advance. The apples were inoculated from 17 to 19 December and estimations of rotted tissue made from 5 to 7 January. Samples of ten apples from each of the nine plots were available for analysis for nitrogen content. The results of these two experiments together with the rate of radial spread of *C. ludibunda* in sterilized apple pulp from each of the samples are given in Table II and expressed graphically in Fig. 2.

Table II

Effect of varied manurial treatment, 1930

No. of plot	Treatment	Mean yield lb.	Mean % weight of scabbed fruit	Average mean radial advance (mm. per day)	Radial spread in apple pulp (mm. in 11 days)	Nitrogen-content (% of fresh weight)
6	K	67·5	15·0	0·082	13·7	0·0185
2	K + P	53·1	8·0	0·118	15·7	0·0233
8*	X*	91·1	10·5	0·130	16·2	0·0254
7	P	71·0	6·2	0·150	16·0	0·0256
9	C	58·6	5·4	0·259	16·5	0·0218
4	N + K + P	94·5	38·7	0·456	26·7	0·0584
3	N + P	58·4	43·7	0·678	29·0	0·0465
5	N	68·1	32·1	1·213	29·0	0·0534
1	N + K	25·7	26·2	1·249	31·5	0·0619

K = muriate of potash; P = superphosphate; C = control; N = nitrogen; X = untreated but does not constitute a control plot.

* This plot contained only fourteen trees and for reasons previously stated was not comparable with other plots. Although not receiving manurial treatment it does not constitute a true control.

It will be seen from Table II that as regards percentage weight of scabbed fruit, radial advance and nitrogen content, the plots fall very clearly into two main classes, (i) those receiving no nitrogen, and (ii) those receiving nitrogen. It is also clear that nitrogenous manuring

lowered the resistance of the fruit to fungal invasion, increased the amount of scabbed fruit and raised the nitrogen content of the fruit (see Fig. 2 A, B and C). The values for nitrogen content run almost parallel with those for radial advance, low nitrogen content being associated with high resistance and vice versa.

It is also interesting to note that the radial spread of *C. ludibunda* in sterilized apple pulp runs almost parallel with the values for radial advance calculated for living tissue.

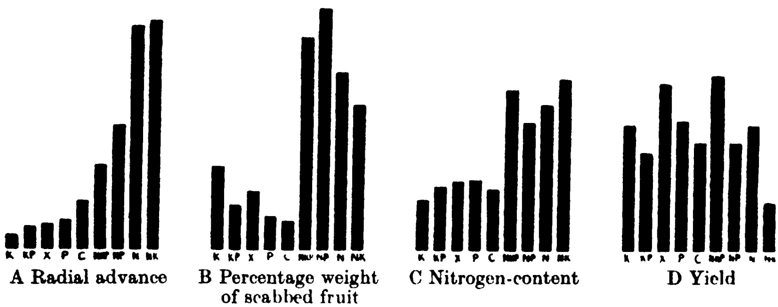

Fig. 2. Effect of varied manurial treatment, 1930.

Statistical analysis of results

A. *Correlations.*

For the purpose of calculating the correlation between the mean values of radial advance and for percentage weight of scabbed fruit the values given for the nine plots in Table II were employed. As has been previously stated, the percentage weight of scabbed fruit was calculated for individual trees from the total yields. Variations in yield were considerable, ranging from 5 to 176 lb. The following result was obtained:

$$r_{SR} = \pm\ 0{\cdot}6395$$

R = radial advance per day, S = percentage weight of scabbed fruit. Reference to Fisher's Table V A (Fisher, 1934) shows that the value obtained falls below the 5 % probability value (0·6664). The correlation is therefore high but not significant. It must be remembered, however, that only nine pairs of values entered into the calculation. The result suggests that fruit which is resistant to an organism which is responsible for causing decay in storage is also resistant to the attacks of the scab organism during growth. It should be pointed out in support of this opinion that the scab lesions were most numerous and largest on apples

from plots which received nitrogenous manure and showed high radial advance.

The rate of radial advance and the percentage weight of scabbed fruit have both been correlated with nitrogen content of the fruit (Table II) with the following results:

$$r_{RN} = +0{\cdot}8571, \quad r_{SN} = +0{\cdot}8292.$$

N = percentage nitrogen in fresh weight of apples. Both these correlations are significant since the value of the coefficient obtained in each case exceeds the 1 % probability value (0·7977).

The coefficient of correlation between the values for mean radial advance and for radial spread of the fungus on sterilized pulp (Table II) has been calculated. The result obtained was as follows:

$$r_{RM} = +0{\cdot}9112,$$

M = radial spread of fungus on sterilized pulp. This correlation is highly significant since the value obtained exceeds the 1 % point (0·7977) and the result indicates that the radial advance of *C. ludibunda* in living apple tissue is directly correlated with the radial spread on sterilized pulp.

B. *Analysis of variance and calculation of standard error.*

Radial advance.

For the purpose of analysis of variance (Fisher, 1934) there were available nine samples, each consisting of forty apples, giving 8 degrees of freedom for treatment and 351 for error. The result of the analysis is given in Table III. The value of Z (2·0754) is more than four times the 1 % probability value and hence is highly significant. This result shows quite definitely that manurial treatment has had a very real effect on resistance of the fruit to invasion by the fungus employed.

Table III

Analysis of variance. Effect of manurial treatment on radial advance, 1930.

	Degrees of freedom	Sum of Squares	S.D.	$\log_e$ S.D.	Z	1 % probability value
Treatment	8	69·52380	2·94	+ 1·07841	2·0754	0·4604
Error	351	47·97815	0·369	− 0·99695	—	—

In order to determine which particular treatments were responsible for the high value of Z, the standard errors for the differences of mean values were calculated. These were not based upon the standard deviation for error given in the analysis of variance (Table III), because it was found that the variance within treatment greatly increased with the

presence of nitrogen as a manurial constituent. Instead, the standard error in each case was calculated from the standard deviation found for the samples compared. The values obtained were as follows:

K *v.* K + P	-0.036 ± 0.011	X *v.* N + P	-0.548 ± 0.086
K *v.* X	-0.048 ± 0.010	X *v.* N	-1.083 ± 0.092
K *v.* P	-0.068 ± 0.010	X *v.* N + K	-1.119 ± 0.105
K *v.* C	-0.177 ± 0.015	P *v.* C	-0.109 ± 0.015
K *v.* N + K + P	-0.374 ± 0.053	P *v.* N + K + P	-0.306 ± 0.054
K *v.* N + P	-0.596 ± 0.089	P *v.* N + P	-0.528 ± 0.089
K *v.* N	-1.131 ± 0.092	P *v.* N	-1.063 ± 0.092
K *v.* N + K	-1.167 ± 0.105	P *v.* N + K	-1.099 ± 0.105
K + P *v.* X	-0.012 ± 0.011	C *v.* N + K + P	-0.197 ± 0.054
K + P *v.* P	-0.032 ± 0.012	C *v.* N + P	-0.419 ± 0.089
K + P *v.* C	-0.141 ± 0.105	C *v.* N	-0.954 ± 0.093
K + P *v.* N + K + P	-0.338 ± 0.054	C *v.* N + K	-0.990 ± 0.105
K + P *v.* N + P	-0.560 ± 0.088	N + K + P *v.* N + P	-0.222 ± 0.103
K + P *v.* N	-1.095 ± 0.092	N + K + P *v.* N	-0.757 ± 0.106
K + P *v.* N + K	-1.131 ± 0.105	N + K + P *v.* N + K	0.793 ± 0.117
X *v.* P	-0.020 ± 0.011	N + P *v.* N	-0.535 ± 0.127
X *v.* C	0.129 ± 0.015	N + P *v.* N + K	0.571 ± 0.137
X *v.* N + K + P	-0.326 ± 0.053	N *v.* N + K	-0.036 ± 0.139

The degree of significance of the difference between various pairs of means is given in Table IV, where the figures in the columns represent the difference between various pairs of mean values divided by the standard error of the difference. In Table IV differences which exceed twice the standard error are regarded as significant. It should be noted that as compared with the control plot nitrogenous manuring has in every case significantly reduced the resistance of the fruit to fungal invasion, while the application of mineral manures without nitrogen has in all cases significantly increased the resistance above that of the control plot.

Table IV

Degree of significance of difference between pairs of mean values for radial advance for samples from plots, 1930

(Figures represent the difference between pairs of mean values divided by the S.E. of the difference.)

	K	K + P	X*	P	C	N + K + P	N + P	N
K + P	3							
X*	4	1						
P	6	2	1					
C	12	9	8	7				
N + K + P	7	6	6	5	3			
N + P	6	6	6	6	4	2		
N	12	11	11	11	10	7	4	
N + K	11	10	10	10	9	6	4	0

* Plot untreated but does not constitute a true control.

Percentage weight of scabbed fruit.

Although it is uncertain whether the application of analysis of variance is strictly valid in the case where estimates of variance are

based on percentage values obtained from uneven yields, nevertheless an analysis has been attempted. For the purpose of the analysis there are eight treatments, plot 8 (see Fig. 2) being eliminated because it contained only fourteen trees. For treatment there were 7 degrees of freedom. Since there were fifteen trees under each treatment error gave 112 degrees of freedom. For error the variance within plots for percentage weights of scabbed fruit calculated for individual trees was determined. The result obtained was as follows:

Table V

Analysis of variance. Effect of manurial treatment on percentage weight of scabbed fruit, 1930.

	Degrees of freedom	Sum of Squares	S.D	$\log_e$ S.D	Z	1% probability value
Treatment	7	24593·55	59·2	4·08092	1 4347	0·4878
Error	112	22316·64	14·1	2·64617	—	—

The value of Z greatly exceeds the 1 % probability value, suggesting that there are real differences in the degree of scab attack between plots receiving different treatments.

The standard errors for the differences between treated plots and the control plot were calculated and these were based on the S.D. found for each pair of samples compared. This method was followed because high variance was associated with nitrogenous manuring and high mean percentage weights of scabbed fruit, whereas low variance was associated with non-nitrogenous or no manuring and low percentage weights of scabbed fruit. The differences between the mean values for treated plots and the control plot are given in Table VI, together with the requisite standard errors. Differences which exceed twice the standard error may be regarded as significant.

Table VI

Differences of mean values for percentage weight of scabbed fruit, 1930

Treatment	Difference from control plot
K	+ **9·6** ±3·1
K + P	+ 2·6 ±1·5
P	+ 0·8 ±1·1
N + K + P	+ **33·3** ±3·7
N + P	+ **38·3** ±5·1
N	+ **26·7** ±4·6
N + K	+ **20·8** ±5·8

The results suggest that five plots including four which received nitrogenous manuring show a significantly higher percentage weight of

fruit attacked by scab than the control plot. In general this result bears out those obtained by the method of correlation.

IX. Results for 1931 and 1932

Field observations

The observations made during 1929 and 1930 on the effects of nitrogen on flowering, foliage and growth of the trees were confirmed. Apples from trees which received nitrogenous manuring were green in colour, and those from trees which received no nitrogenous manure were blushed but the distinctions were not so clear as in 1930.

The yield of fruit in both years was very small and, although the crop borne by each tree and the percentage showing established infection with *Venturia inaequalis* were recorded, it is considered that no useful purpose would be served by a statement of results.

Observations on resistance to fungal invasion

In 1931 a sample of fifteen apples from each of the thirty plots into which the orchard was divided in that year were available for biological work. These were inoculated 23–27 November and estimations of the rotted tissue were carried out 21 days from the time of inoculation. Samples of six to fifteen apples from each of these plots were analysed for nitrogen content in 1931. The results of these experiments are given in Table VII. It should be observed that in most cases plots which received nitrogenous manuring in either 1930 or 1931 gave high values for nitrogen content and radial advance.

In 1932 a sample of twenty apples was selected to represent the population of apples produced by trees which had received nitrogenous manuring either alone or in addition to other fertilizers, and another sample of equal number to represent the population of apples borne by trees which had received no nitrogenous manure, but some of which had received a dressing of potash or phosphate. Only plots which had received exactly the same treatment since the commencement of the experiment in 1929 were represented in the samples. The apples were inoculated on 10 January. The data for average mean radial advance for these samples are given in Table VIII side by side with the average means of comparable samples inoculated in 1930 and 1931. It should be noted that in all three years the radial advance is much higher for samples from plots receiving nitrogenous manuring than from plots which did not receive nitrogen.

Table VII

Effect of varied manurial treatment on apple fruit, 1931

Plot		Manurial treatment 1929 and 1930	Manurial treatment 1931	Average mean radial advance (mm. per day)	Nitrogen content % fresh weight
Block I	1	N	—	0·370	0·0444
	2	N	N	0·551	0·0710
	3	N	2P	1·027	0·0719
	4	N	2K	0·365	0·0550
Block II	5	K	—	0·610	0·0507
	6	K	K	0·553	0·0579
	7	K	N	0·802	0·0603
	8	K	2P	0·094	0·0279
Block III	9	P	—	0·239	0·0380
	10	P	P	0·173	0·0424
	11	P	2K	0·181	0·0402
	12	P	N	0·789	0·0493
Block IV	13	N + K	—	0·401	0·0633
	14	N + K	N + K	1·168	0·0835
	15	N + K	2P	0·808	0·0562
	16	N + K	2K	0·655	0·0697
Block V	17	N + P	—	0·326	0·0432
	18	N + P	N + P	1·292	0·0550
	19	N + P	2K	1·380	0·0516
	20	N + P	2P	1·135	0·0705
Block VI	21	K + P	—	0·152	0·0439
	22	K + P	K + P	0·388	0·0374
	23	K + P	2K	0·214	0·0388
	24	K + P	N	0·741	0·0540
Block VII	25	N + K + P	—	0·824	0·0723
	26	N + K + P	N + K + P	1·303	0·0885
	27	N + K + P	2P	1·092	0·0601
	28	N + K + P	2K	0·580	0·0643
	29	Control	Control	0·210	0·0343
	30	Control	F.Y.M.	0·150	0·0367

Table VIII

Average mean radial advance (mm. per day), 1930–2

Year	No. of apples in each sample	No. of trees represented by each sample	Average mean radial advance (mm. per day) for sample from trees receiving nitrogenous manuring	Average mean radial advance (mm. per day) for sample from trees receiving no nitrogenous manuring	Difference
1930	160	64	0·889	0·153	**0·736** ±0·051
1931	60	20	1·08	0·33	**0·75** ±0·095
1932	20	20	0·89	0·54	**0·35** ±0·110

Statistical analysis of results

It has been possible to make only one correlation, viz. that between radial advance and nitrogen content of the fruit using the values given in Table VII. The result was as follows:

$$r_{RN} = +0{\cdot}7277.$$

This correlation is significant since the value obtained greatly exceeds the 1 % probability value (0·4641).

Analysis of variance was applied to the data of radial advance for samples from plots each of which received the same treatment from year to year during the experiment. For this purpose there were available eight samples each consisting of fifteen apples, giving 7 degrees of freedom for treatment, and 112 for error. The result of the analysis is given in Table IX. The value of Z greatly exceeds the 1 % probability value, again indicating that manuring has exercised a real effect on the resistance of the fruit to fungal invasion.

Table IX

Analysis of variance. Effect of manurial treatment on radial advance, 1931.

	Degrees of freedom	Sum of Squares	S.D.	$\log_e$ S.D.	Z	1 % probability value
Treatment	7	23·8785	1·84	+0·60977	1·3863	0·4878
Error	112	23·7272	0·46	−0·77652	—	—

The standard errors for the differences in radial advance between each treated plot dealt with in the analysis of variance and the control plot, based on the standard deviation estimated for the requisite pairs of samples are as follows: C *v.* P, +0·037 ± 0·106; C *v.* K + P, −0·178 ± 0·164; C *v.* N, −0·341 ± 0·176; C *v.* K, **−0·343** ± 0·167; C *v.* N + K, **−0·958** ± 0·170; C *v.* N + P, **−1·082** ± 0·152 and C *v.* N + K + P, **−1·093** ± 0·162. It is seen that the high value of Z is mainly conditioned by the three treatments N + K, N + P and N + K + P which gave fruit of very low resistance.

Similar calculations were made for plots which were untreated in 1931 but which had been treated in 1929 and 1930 with the following results: C *v.* K + P, +0·058 ± 0·104; C *v.* P, −0·029 ± 0·129; C *v.* N + P, −0·116 ± 0·150; C *v.* N, −0·160 ± 0·129; C *v.* N + K, −0·191 ± 0·149; C *v.* K, **−0·400** ± 0·180 and C *v.* N + K + P, **−0·614** ± 0·182. In this case only two differences are significant, viz. those where K and N + K + P were used as fertilizers in 1929 and 1930.

It must be remembered that the results for 1931 given above are based on fruit in a year when the yield was negligible from a commercial point of view.

X. Discussion

This investigation was primarily undertaken with the object of determining the effect of manurial treatment upon the incidence of apple scab, but its scope was subsequently extended so as to embrace a study of the effects of treatment upon resistance of the fruit to fungal invasion

as measured by the radial advance of *C. ludibunda*, as well as observations upon growth, yield, bloom, fruit colour and nitrogen content.

Wallace (1933) has shown by pot experiments that the omission of nitrogen from nutrient solutions supplied to apple trees brings about a delay in the opening of blossom buds, a reduction of blossom formation, a reduction in the amount of foliage and also leads to the production of fruits which are often highly coloured or of chlorotic appearance. These observations have been confirmed by the present investigation where it was known that the soil was deficient in nitrogen, and it was further shown that by nitrogenous manuring such conditions could be rectified.

Unfortunately a good crop was obtained only in one year (1930), and since in the other three years very poor crops were produced the greater part of the results presented here are for one year only. Studies in the resistance of the fruit to fungal invasion were, however, carried out during three years, but during one year (1932) the samples available were very small.

The results in 1930 suggest that the fruit from the trees receiving nitrogen yielded a definitely higher percentage weight of apples attacked by scab than those receiving no nitrogen. The true position as regards incidence of scab is not quite reflected by the figures in Table II, since the scab lesions on apples from trees receiving no nitrogen were small; those on fruit from trees receiving nitrogen being larger and more numerous. Potash manuring may also be responsible for an increase in the percentage weight of fruit showing established infection by scab. This result obtained by potash manuring points in the same direction as that obtained by Grubb (1930) at East Malling.

Manurial treatment has been shown to exercise a great effect on the resistance of the fruit to fungal invasion by *C. ludibunda*. In 1930 it was found that the mean rate of growth of the fungus in the samples from plots receiving nitrogenous manuring was in every case much higher than in apples from plots which received non-nitrogenous or no manure. For example, the mean growth rate in the sample from the plot treated with both nitrogen and potash was actually more than fifteen times that in the sample from the plot which received potash only, and almost five times that in the sample from the control plot. In 1931 and 1932 when the crops borne were very small it is interesting to note that even under these conditions and where only small samples for each treatment were available it was again possible to demonstrate that nitrogenous manure lowered the resistance of the fruit.

The application of potash, phosphate, or of both brought about a

definite reduction in the rate of radial advance in 1930. The mean rate in the sample from the control plot was more than three times that in the apples from the plot treated with potash. The effect of manuring with potash or phosphate obtained in 1930 was not repeated in 1931. Since the samples available in the latter year were small it is doubtful whether any real confidence can be placed in the fact that in 1931 treatment with potash appeared to lower the resistance. However, it has been shown in another investigation (Horne, 1935–6) that manuring with potash in one year lowered resistance and in the following year increased the resistance of the apples borne on the same trees. In 1932 the samples available were so small that only the effect of nitrogenous as opposed to non-nitrogenous or no manuring was investigated.

Great confidence can be placed in the results for radial advance obtained in 1930 because in that year the yield was high and large samples were available for experimental work. Further, it has been shown that the values obtained in that year for the rate of radial spread of *C. ludibunda* in sterilized pulp from each of the samples ran parallel with the values for its rate of radial advance in living tissues. This close relationship between the growth rates in dead and living tissues indicates that the rate of advance of the fungus in the living fruit is governed largely by the chemical composition of the fruit. This is in agreement with the work of Seth (1934) who showed that the rate of spread of the fungus on artificial medium is influenced by the chemical composition of the medium.

The only information available with regard to the effect of manuring on chemical composition of the fruit is that for nitrogen content. This shows that nitrogenous manuring greatly increases the nitrogen content, and it has been proved that in each of the two years for which the values are available that nitrogen content and radial advance are directly correlated.

The suggestion of a marked relationship in 1930 between radial advance and percentage weight of scabbed fruit is of interest. In another orchard in Northern Ireland it has since been shown that there was in one year a high but not significant correlation between radial advance and percentage weight of scabbed fruit (unpublished data).

It appears from the results presented that the nitrogen content of the fruit varies in different seasons and that the higher the yield the lower the nitrogen content. The relation between yield and nitrogen content is reflected in the rate of fungal invasion; low yields being associated with low resistance and high nitrogen content, and high yields

with high resistance and low nitrogen content. Further work along these lines is desirable and although these observations hold for the experimental orchard and for the years under review, it is uncertain whether or not they are of general application.

During the investigation it was observed in all three years that, when the figures for radial advance were studied, high variance within samples was associated with nitrogenous manuring, whereas low variance was associated with non-nitrogenous or no manuring. It may be suggested that high variance in the samples from plots heavily manured with nitrogen in a readily available form may be due, to some extent, to the rapid and uneven absorption of the nutrient by the tree, although the manure was spread as evenly as possible.

Wallace (1933) has pointed out that nitrogen starvation is of frequent occurrence under grass systems of orchard cultivation. The present work demonstrates that in rectifying this condition care must be exercised in the application of nitrogenous fertilizers or the quality of the fruit may suffer very severely; the results also suggest that such manuring may render the control of scab more difficult. It has been indicated also that under Northern Ireland conditions the carrying out regularly of an efficient spraying programme for the control of diseases and pests is a very important factor in the production of good crops.

XI. Summary

1. Manurial treatment exercises a strong influence on the growth of apple trees, their time of blossoming, the amount of bloom, the foliage colour and the fruit colour. The effect is mainly due to nitrogen which induces increased growth, earlier flowering, more bloom, greener foliage and softer and greener fruit. Potash and phosphate have little effect on the growth of the trees, or on blossoming or foliage, but favour the production of more highly coloured fruit.

2. Nitrogenous manuring increases the nitrogen content of the fruit. Thus in 1930 the mean nitrogen content for the samples from plots receiving and not receiving nitrogen, was 0·0229 and 0·0550 respectively. In the following year the results for similarly treated plots were 0·0430 and 0·0745 respectively. Considering the results obtained in the years in question the mean nitrogen content for 1930 when there was a good yield was 0·0372 in contrast with 0·0587 for 1931 when the yield was poor. The control plot alone gave values of 0·0218 and 0·0343.

3. In 1930 the rates of radial advance (in mm. per day) of *Cytosporina ludibunda* for samples from plots receiving different treatments were

K, 0·082; K+P, 0·118; X, 0·130; P, 0·150; C, 0·259; N+K+P, 0·456; N+P, 0·678; N, 1·213; and N+K, 1·249. The rate for the sample from the plot treated with N+K is fifteen times that calculated for the apples from the plot treated with K. The effect of nitrogenous as compared with non-nitrogenous or no manuring has been studied in each of three years. The mean values for radial advance obtained for samples from plots receiving no nitrogen were 1930, 0·153; 1931, 0·33; 1932, 0·54, while for samples from plots receiving nitrogenous manuring the values were 1930, 0·889; 1931, 1·08; and 1932, 0·89. The values obtained in 1930 receive confirmation from observations on the radial spread of the fungus on the sterilized tissue from apples produced on trees in the various plots. The value $r_{RM} = +0{\cdot}9112$ was obtained for the correlation between radial advance (R) and radial spread (M). In 1930 the rate of growth of the fungus in the sample from the plot treated with K was one-third of that in the sample from the control plot.

4. Radial advance is correlated with nitrogen content (N) of the fruit and this relationship is shown by the following values for the correlation coefficient: 1930, $r_{RN} + = 0{\cdot}8571$; 1931, $r_{RN} = +0{\cdot}7277$.

5. Nitrogenous manuring increases the intensity of the attack of *Venturia inaequalis* on the fruit in the orchard. Evidence is produced in support of the suggestion that fruit resistant to attack by *V. inaequalis* may also be resistant to invasion by a rot producing fungus in storage. The value of the correlation between radial advance (R) and the percentage weight of scabbed fruit (S) was $r_{RS} + = 0{\cdot}6395$. The percentage weight of scabbed fruit was shown to be correlated with the nitrogen content of the fruit and the value of the coefficient obtained was $r_{SN} = +0{\cdot}8292$.

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STUDIES IN POTATO STORAGE

II. INFLUENCE OF (1) THE STAGE OF MATURITY OF THE TUBERS AND (2) THE STORAGE TEMPERATURE FOR A BRIEF DURATION IMMEDIATELY AFTER DIGGING, ON PHYSIOLOGICAL LOSSES IN WEIGHT OF POTATOES DURING STORAGE

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(With 3 Text-figures)

In paper I (Singh & Mathur, 1937) of this series attention was given to the problem of maturity in potatoes, and it was shown that, while developing on the vine, the potato tuber passes through three more or less well-defined stages designated as adolescence, maturity, and ripening. In extending the work particular attention was given to a study of the influence of developmental stage of the tubers at the time of harvest on physiological losses during storage under different conditions. Information was sought regarding the extent to which the chief maturing processes in the tubers might continue in storage. Will potatoes, immature yet large enough for seed, attain during storage the percentage composition and the physiological condition characteristic of the tubers allowed fully to mature on the vine?

At the time of harvest the periderm layer of the tuber is only partly formed and the process of periderm formation continues for some time during storage, depending upon environmental conditions. Several investigators have studied the anatomy of the periderm of potato and the conditions conducive to rapid cork formation. They generally agree that the periderm formation is hastened by high temperature and high humidity. It is desirable to hasten the process of periderm formation in order to reduce the loss in weight due to increased transpiration and respiration during storage. Since the periderm layer when completely formed is impermeable to water and gases it might be inferred, as indeed it has been suggested by several investigators, that the total carbon dioxide production during metabolism will not be the same as its

superficial evolution, and the internal atmospheres of tubers will, in consequence, be characterized by fairly high concentrations of carbon dioxide.

A study of the storage-room atmosphere surrounding the bin of potatoes is likely to throw light on such questions as limiting the size of the bins, as well as the number and duration of aerations that are necessary for successful storage. Results of potato-storage experiments in which small lots of tubers have been stored may be of little value in solving the problems in the storage house where the potatoes are usually stored in bulk. A small lot of tubers exposed to the air on all sides will have an entirely different environment and will not react with regard to its surroundings in the same way as will those stored in bulk.

EXPERIMENTAL PROCEDURE

The experimental crop was raised from seed belonging to the variety Farrukhabad. During 1934–5, beginning when the vines were in bloom, four to six typical hills were dug at intervals of 3 days and the tubers divided into three lots, each consisting of an equal number of tubers of approximately the same size. One lot was sampled immediately for respiration measurements and chemical analysis (the results have already been presented in the first paper (Singh & Mathur, 1937), while the other two were placed in temporary storage at two fairly constant temperatures of 7 and 18° C. for 10–12 days. At the expiry of the preliminary storage periods, the two lots of tubers were placed in the same permanent storage at $13 \pm 0{\cdot}9°$ C. The storage lots were all taken out for experimentation on 25 May 1935.

Determinations of respiration intensity and chemical constituents were made according to methods already given (Singh & Mathur, 1937).

RESULTS

Fig. 1 shows that, in general, the tubers that had been in a temporary storage at 7° C. before being permanently stored at 13° C. possess a higher respiration intensity than the ones pre-stored at 18° C. It was also observed that the tubers pre-stored at 7° C. terminated their period of dormancy sooner and exhibited a more extensive sprout growth than the comparable lots stored at the higher temperature for a brief duration. This fact probably accounts for the greater respiration rate of the lot pre-stored at 7° C.

It is evident from Tables I and II that at the end of the storage period the lots pre-stored at 18° C. possess in general a higher percentage of

total sugars than those temporarily stored at 7° C. The greatest loss in the total sugar content occurred in the adolescent tubers, although they still contained the highest percentage of total sugars at the end of the storage period. In contrast to total sugar content the quantity of reducing sugars increases during storage in adolescent tubers under both

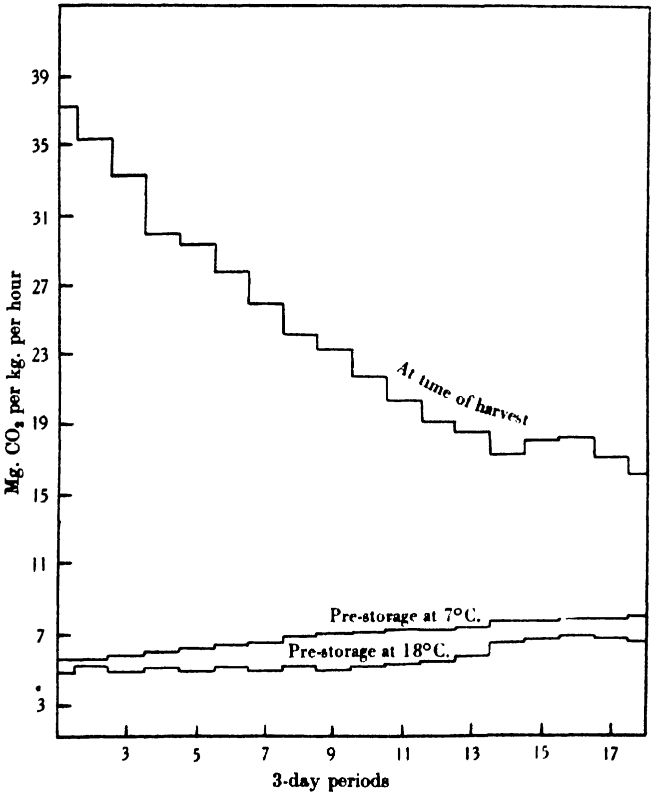

Fig. 1. Respiration intensity of potatoes of different developmental stages at time of harvest and at the end of storage period.

the pre-treatments. In the later harvested, mature tubers, however, the percentage of reducing sugars decreases during storage. Generally speaking, the potatoes stored for a brief period at 7° C. possess a higher reducing sugar content at the end of the permanent storage than those temporarily stored at 18° C. In contrast to total sugars the percentage of crude fibre increases during storage irrespective of the pre-treatment.

Table I

Percentage composition of potatoes of different developmental stages at time of harvest and at the end of storage period

		Total sugars			Reducing sugars		
			At the end of storage period			At the end of storage period	
Date of harvest	Develop-mental stage	At time of harvest	Pre-storage at 7° C.	Pre-storage at 18° C.	At time of harvest	Pre-storage at 7° C.	Pre-storage at 18° C
27. xi. 34	Adolescent	1·03	0·39	0·41	0·208	0·272	0·232
30. xi. 34		1·02	0·36	0·39	0·201	0·271	0·220
3. xii. 34		1·03	0·35	0·37	0·198	0·272	0·219
6. xii. 34		1·04	0·30	0·35	0·147	0·271	0·215
9. xii. 34		0·97	0·28	0·33	0·132	0·221	0·199
12. xii. 34	Mature	0·88	0·27	0·32	0·122	0·217	0·197
15. xii. 34		0·68	0·26	0·31	0·112	0·208	0·188
18. xii. 34		0·57	0·23	0·30	0·109	0·197	0·176
21. xii. 34		0·49	0·21	0·27	0·107	0·096	0·075
24. xii. 34		0·31	0·18	0·25	0·103	0·078	0·060
27. xii. 34		0·28	0·18	0·23	0·101	0·064	0·051
30. xii. 34		0·27	0·17	0·20	0·097	0·059	0·049
2. i. 35		0·26	0·16	0·19	0·087	0·089	0·057
5. i. 35	Ripe	0·29	0·27	0·26	0·096	0·102	0·081
8. i. 35		0·28	0·23	0·25	0·098	0·106	0·089
11. i. 35		0·28	0·23	0·23	0·088	0·110	0·087
14. i. 35		0·27	0·22	0·20	0·049	0·049	0·036
17. i. 35		0·25	0·21	0·22	0·048	0·048	0·036

Table II

Percentage composition of potatoes of different developmental stages at time of harvest and at the end of storage period

		Sucrose			Starch		
			At the end of storage period			At the end of storage period	
Date of harvest	Develop-mental stage	At time of harvest	Pre-storage at 7° C.	Pre-storage at 18° C.	At time of harvest	Pre-storage at 7° C.	Pre-storage at 18° C.
27. xi. 34	Adolescent	0·789	0·138	0·139	11·01	11·56	11·89
30. xi. 34		0·744	0·130	0·137	11·09	11·84	12·21
3. xii. 34		0·837	0·129	0·134	11·75	11·91	12·85
6. xii. 34		0·903	0·127	0·133	11·79	11·92	12·86
9. xii. 34		0·837	0·126	0·129	12·23	12·96	12·98
12. xii. 34	Mature	0·673	0·120	0·128	13·75	13·11	13·37
15. xii. 34		0·567	0·117	0·127	14·01	13·87	13·92
18. xii. 34		0·327	0·117	0·126	14·07	13·99	14·03
21. xii. 34		0·295	0·115	0·125	14·61	14·01	14·46
24. xii. 34		0·197	0·114	0·124	15·02	14·91	14·92
27. xii. 34		0·184	0·113	0·122	14·91	14·77	14·87
30. xii. 34		0·139	0·121	0·131	14·79	13·92	14·15
2. i. 35		0·147	0·172	0·136	14·29	13·88	14·06
5. i. 35	Ripe	0·189	0·149	0·156	14·01	13·92	14·02
8. i. 35		0·187	0·139	0·156	13·93	14·81	14·93
11. i. 35		0·187	0·130	0·147	13·44	14·69	15·01
14. i. 35		0·145	0·129	0·144	13·45	14·92	15·02
17. i. 35		0·144	0·127	0·141	13·49	14·92	15·01

Table III

Percentage composition of potatoes of different developmental stages at time of harvest and at the end of storage period

Date of harvest	Developmental stage	Moisture: At time of harvest	Moisture: At the end of storage period: Pre-storage at 7° C.	Moisture: At the end of storage period: Pre-storage at 18° C.	Total nitrogen: At time of harvest	Total nitrogen: At the end of storage period: Pre-storage at 7° C.	Total nitrogen: At the end of storage period: Pre-storage at 18° C.
27. xi. 34	Adolescent	83·67	80·21	81·31	0·29	0·38	0·34
30. xi. 34		83·29	80·07	81·57	0·29	0·38	0·36
3. xii. 34		83·13	79·87	81·69	0·30	0·39	0·37
6. xii. 34		82·69	79·76	81·81	0·31	0·37	0·33
9. xii. 34		82·22	79·23	80·75	0·31	0·36	0·34
12. xii. 34	Mature	80·97	79·06	80·73	0·30	0·30	0·30
15. xii. 34		80·77	78·92	80·10	0·30	0·30	0·32
18. xii. 34		80·73	78·91	79·26	0·37	0·35	0·34
21. xii. 34		79·97	78·92	78·99	0·38	0·36	0·35
24. xii. 34		79·38	78·21	78·32	0·39	0·37	0·34
27. xii. 34		79·37	78·82	78·89	0·38	0·38	0·36
30. xii. 34		80·29	78·61	78·87	0·40	0·39	0·38
2. i. 35		80·23	78·42	79·62	0·40	0·39	0·39
5. i. 35	Ripe	80·73	79·12	79·67	0·42	0·47	0·43
8. i. 35		81·04	78·81	79·69	0·43	0·48	0·43
11. i. 35		81·07	78·87	79·73	0·38	0·43	0·40
14. i. 35		80·71	78·81	79·21	0·37	0·42	0·39
17. i. 35		80·93	78·80	79·07	0·38	0·42	0·38

Table IV

Percentage composition of potatoes of different developmental stages at time of harvest and at the end of storage period

Date of harvest	Developmental stage	Ash: At time of harvest	Ash: At the end of storage period: Pre-storage at 7° C.	Ash: At the end of storage period: Pre-storage at 18° C.	Crude fibre: At time of harvest	Crude fibre: At the end of storage period: Pre-storage at 7° C.	Crude fibre: At the end of storage period: Pre-storage at 18° C.
27. xi. 34	Adolescent	0·93	1·22	1·20	0·35	0·48	0·42
30. xi. 34		0·92	1·21	1·23	0·34	0·46	0·42
3. xii. 34		0·93	1·22	1·19	0·32	0·45	0·41
6. xii. 34		0·91	1·11	1·11	0·31	0·44	0·40
9. xii. 34		0·91	1·08	1·09	0·32	0·41	0·39
12. xii. 34	Mature	0·92	1·07	1·08	0·32	0·39	0·38
15. xii. 34		0·92	1·08	1·07	0·32	0·38	0·36
18. xii. 34		0·97	1·09	1·06	0·31	0·38	0·35
21. xii. 34		0·99	1·07	1·08	0·31	0·38	0·32
24. xii. 34		1·01	1·06	1·07	0·31	0·38	0·31
27. xii. 34		1·01	1·22	1·07	0·33	0·39	0·30
30. xii. 34		1·01	1·21	1·08	0·31	0·40	0·31
2. i. 35		1·02	1·17	1·09	0·32	0·38	0·31
5. i. 35	Ripe	1·02	1·16	1·16	0·35	0·36	0·30
8. i. 35		1·02	1·15	1·17	0·34	0·35	0·31
11. i. 35		1·03	1·13	1·17	0·32	0·35	0·31
14. i. 35		1·03	1·12	1·21	0·31	0·33	0·31
17. i. 35		1·03	1·19	1·22	0·33	0·31	0·31

Table V

Percentage values of losses due to respiration and transpiration in 10-lb. lots of tubers during the storage period

Date of digging	Developmental stage	Pre-storage at 7° C. Shrinkage by water loss	Pre-storage at 7° C. Shrinkage by respiration	Pre-storage at 18° C. Shrinkage by water loss	Pre-storage at 18° C. Shrinkage by respiration
27. xi. 34	Adolescent	14·94	1·01	11·95	0·98
30. xi. 34		13·30	0·97	11·23	0·88
3. xii. 34		12·14	0·92	10·22	0·87
6. xii. 34		12·00	0·93	9·53	0·78
9. xii. 34		11·29	0·96	8·60	0·67
12. xii. 34	Maturity	10·52	0·87	7·68	0·66
15. xii. 34		7·91	0·77	7·44	0·63
18. xii. 34		6·63	0·63	6·50	0·52
21. xii. 34		6·32	0·61	6·14	0·49
24. xii. 34		6·19	0·54	5·60	0·41
27. xii. 34		5·61	0·51	4·80	0·39
30. xii. 34		5·53	0·49	4·58	0·39
2. i. 35		5·53	0·44	4·08	0·35
5. i. 35	Ripe	4·56	0·39	3·63	0·34
8. i. 35		4·39	0·44	3·54	0·34
11. i. 35		4·20	0·43	3·35	0·37
14. i. 35		3·79	0·42	2·78	0·36
17. i. 35		3·54	0·41	2·32	0·27

Table VI

Physiological loss in weight of tubers at different depths in the bin

Date of digging	Developmental stage	Pre-storage at 7° C. Top layer	Pre-storage at 7° C. Middle layer	Pre-storage at 7° C. Bottom layer	Pre-storage at 18° C. Top layer	Pre-storage at 18° C. Middle layer	Pre-storage at 18° C. Bottom layer
27. xi. 34	Adolescent	10·29	15·95	19·52	8·68	12·93	16·97
30. xi. 34		10·01	14·27	18·53	8·12	12·11	16·12
3. xii. 34		9·79	13·06	16·33	7·29	11·09	15·08
6. xii. 34		9·29	12·93	16·57	6·99	10·31	14·37
9. xii. 34		8·11	12·25	16·12	6·23	9·29	12·28
12. xii. 34	Mature	7·92	11·39	15·39	5·95	8·34	11·37
15. xii. 34		5·65	8·68	11·69	5·29	8·07	11·08
18. xii. 34		4·27	7·26	10·27	5·09	7·02	9·08
21. xii. 34		3·91	6·39	9·96	4·98	6·63	8·79
24. xii. 34		3·46	6·73	9·62	4·91	6·01	8·09
27. xii. 34		3·32	6·12	9·13	4·82	5·19	6·79
30. xii. 34		3·17	6·02	9·01	3·92	4·97	5·98
2. i. 35		3·09	5·97	7·99	3·88	4·43	5·46
5. i. 35	Ripe	2·98	4·95	6·97	3·21	3·97	4·97
8. i. 35		2·97	4·83	6·84	2·99	3·88	4·91
11. i. 35		2·82	4·63	6·63	2·88	3·72	4·73
14. i. 35		2·76	4·21	6·21	2·83	3·14	4·71
17. i. 35		2·55	3·95	5·93	2·01	2·39	3·91

Of potatoes of all developmental stages the greatest loss in the sucrose content occurs in the earlier harvested, adolescent tubers. At the end of the storage period the sucrose content in all the lots was practically the same irrespective of the developmental stage of the tuber and the

pre-storage temperature. Nevertheless, a greater loss of sucrose was observed in the tubers pre-stored at 7° C. than in the lots placed at 18° C. for a brief duration previous to permanent storage. During the time when the tubers are maturing on the vines a slight increase in the sucrose content is noticed. It is significant to note that potatoes belonging to this lot continued to be distinguished by a higher sucrose content up to the end of the storage period. In contrast to the data obtained for sucrose, the quantity of starch increases in the adolescent tubers during storage under both the pre-treatments. At any rate, a distinct increase in the starch content is discernible in ripe potatoes at the end of the storage period. This is true of both the pre-treatments, though a greater starch accumulation took place in the lot temporarily stored at 18° C.

Tables III and IV show that the moisture content of potatoes decreases slightly during permanent storage irrespective of the pre-treatment. Greater loss of water occurred in the tubers pre-stored at 7° C. as compared to those maintained at 18° C. previous to permanent storage. The percentage of total nitrogen increases during storage in adolescent as well as ripe tubers under both the pre-treatments. In general, the potatoes pre-stored at 7° C. for a brief duration possess a higher total nitrogen content at the end of the storage period than the lots temporarily placed at the higher temperature. Moreover, a gradual increase in the ash content occurs with the advancement of maturity of the tubers. In the adolescent and mature potatoes this process continues in the storage (irrespective of the pre-treatment), so that, by the end of the storage period, the percentage ash in all the lots is practically the same and is equal to that found in the ripe tubers immediately after digging.

It is evident from Table V that the loss in weight of potatoes due to respiration is very small in comparison with that caused by evaporation of water. In the adolescent tubers the loss in weight during storage is high, and the value for total loss decreases with increasing maturity of the tubers. Tubers stored for 10–12 days at 18° C. lost considerably less weight during subsequent storage than did those stored for the same length of time at 7° C. previous to permanent storage.

The data concerning the effect of depth of piling potatoes on the loss in weight of tubers at different depths are presented in Table VI. Potatoes were placed in bins 4 ft. long and 3 ft. wide and were piled to a depth of 3½ ft. in three layers, each layer 14 in. deep and separated from the other by ½ in. mesh wire netting. The data show that comparatively less shrinkage occurs in the top layer than in the middle and the bottom ones.

While the work was in progress it was found desirable to study the effects of pre-storage temperature on the rapidity and the extent of suberization and wound-periderm formation. The material for histological examinations was selected from mature tubers which were divided into

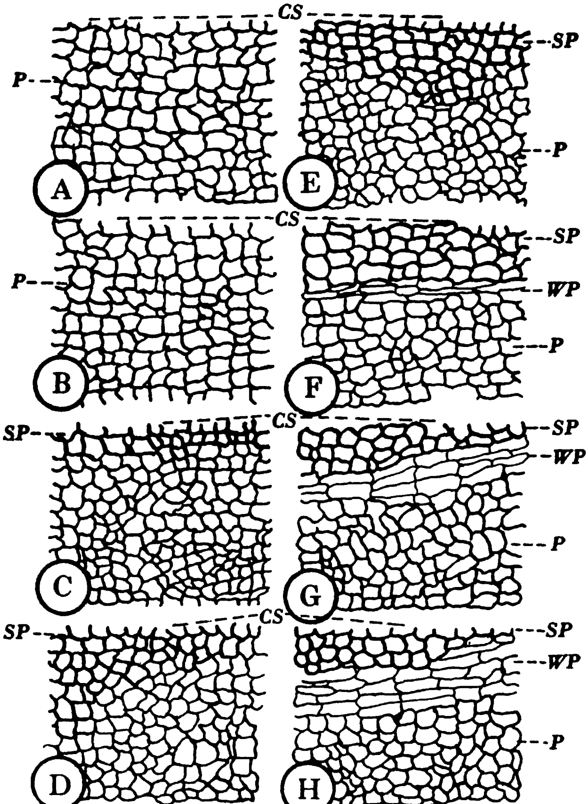

Fig. 2. Camera lucida drawings of sections of periderm of potatoes placed at 7° C. and and 18° C. for 12 days. *CS*, cut surface; *SP*, suberized parenchyma; *WP*, wound periderm and *P*, parenchyma. × 50.

two lots, one of which was placed in storage at an average temperature of 7° C. and the other at 18° C. for 12 days. Each day during the pre-storage period several wounded potatoes were selected from each of the storage temperatures and freehand sections were cut and examined microscopically for the appearance of suberization and periderm formation in the wounded areas. Camera lucida drawings of the suberized and

periderm layers of tubers in storage at the two temperatures are shown in Fig. 2. Suberization first made its appearance on the ninth day in tubers stored at 7° C., whereas appreciable suberization was observed in the lot stored at 18° C. as early as the third day. Wound periderm was

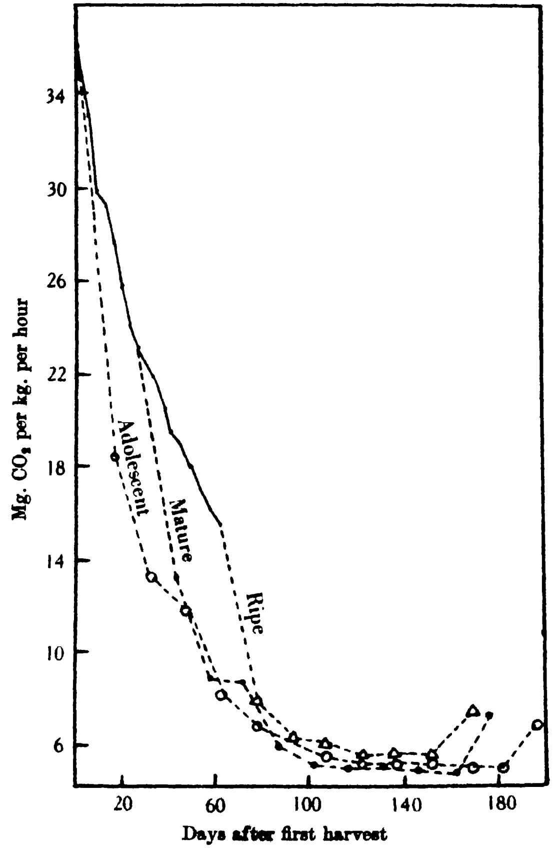

Fig. 3. Composite diagram showing the respiration intensity of potatoes during adolescence, maturity and ripening (continuous curve) and also the respiration rates of tubers of the same developmental stages during storage (discontinuous curves).

first noted on the sixth day of storage at 18° C., while the tubers stored at 7° C. had no periderm layer even at the end of 12 days of storage. Fig. 3 is a composite diagram constructed from data obtained during 1934–5 as well as 1935–6. The bold continuous curve represents the rate of respiration of developing tubers during the growing season, and the

discontinuous curves represent the respiration rates of adolescent, mature and ripe tubers dug at intervals of 21 days and immediately placed in storage at $13 \pm 0{\cdot}8°$ C. The decrease in the respiration rate is much more rapid during storage than with those potatoes that remain in the soil for the same length of time. Another interesting feature brought out by Fig. 3 is that, starting from the day of harvest, the initiation of sprouting is hastened more and more with the increase of the time during which the tubers are allowed to remain in the soil. In other words, if potatoes of all developmental stages be stored on the same day the immature ones will keep in storage the longest. To take a concrete example, counting from the first day of harvest the adolescent tubers begin to sprout 20–40 days later than the mature and ripe ones. This finding, together with our observation that mature potatoes possess at the end of the storage period practically the same percentage composition as the ripe ones, indicates the superiority of the former over the latter for seed purposes.

Appleman *et al.* (1928) demonstrated that during the storage of potatoes the loss in weight due to respiration is considerably less than that due to transpiration. Smith (1934) has brought forward evidence which shows that a large amount of water may be lost from the tubers before the healing of the wounds inflicted during harvesting is completed. Artschwager (1927) came to the conclusion that a temperature of 69·8° F. and a relative humidity of 95% are the lowest optimum conditions for rapid wound-periderm formation. Weiss *et al.* (1928) have shown that, at temperatures below 50° F., even at favourable humidity, the tubers are not able to form a protective layer which will exclude the more virulent rot organisms.

A greater loss in weight of adolescent tubers during storage in comparison with mature and ripe ones appears to be due to the fact that, in the former, the periderm layer is incompletely formed and the tubers are, in consequence, more susceptible to injuries during harvesting. Evidently a greater loss of moisture and carbon dioxide will occur from heavily wounded immature tubers. Smith (1929) showed that the more immature the tubers are when harvested the greater is their rate of respiration. This evidence likewise helps to explain the large amount of shrinkage from the adolescent potatoes.

The lessening of shrinkage after a pre-storage at 18° C. as contrasted with a temporary storage at 7° C. may be explained on the assumption that the process of wound-periderm formation is hastened at the higher temperature thus curtailing the loss in weight during subsequent storage.

Obviously the lower temperature retards the process of suberization and periderm formation in the wounded areas, and therefore the period of rapid evaporation and high rate of respiration is prolonged. This results in a greater loss in weight of tubers pre-stored at the lower temperature. It is desirable to hasten the process of wound-periderm formation in order to reduce the loss in weight by increased evaporation and respiration from the wounded areas or from the uninjured parts on which periderm formation is not yet complete.

Summary

1. Tubers stored for 10–12 days at 18° C. lost considerably less weight during subsequent storage than those pre-stored at 7° C. previous to permanent storage. This emphasizes the importance of pre-storing potatoes for a brief duration at a higher temperature preparatory to permanent cold storage.

2. In the adolescent tubers the loss in weight during storage is high and decreases with increasing maturity of the tubers, the value for the total loss being about the same in mature and ripe tubers.

3. Although the magnitude of shrinkage during storage of mature and ripe potatoes is practically the same, the former are superior to the latter in that they keep longer in storage without sprouting.

4. During storage the loss in weight of potatoes due to respiration is very small in comparison with that caused by evaporation of water.

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STUDIES IN POTATO STORAGE

III. RESPIRATION OF POTATO TUBERS DURING STORAGE

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(With 3 Text-figures)

IN a recent communication (Singh & Mathur, 1937) from this laboratory it was shown that during its development the potato tuber passes through three more or less well-defined stages designated as adolescence, maturity and ripening. In this connexion an important question that suggested itself was whether potatoes belonging to the three above-mentioned stages are distinguishable in their respiratory behaviour during the storage period. If specific differences occur in the respiratory behaviour of potatoes of different developmental stages such studies may prove fruitful in solving certain problems of gas-storage of tubers, for success in storing tubers in various gaseous mixtures depends upon the correctness with which the rate of respiration during the subsequent period of storage can be predicted. These considerations led to experiments, the results of some of which are briefly described.

METHODS

The tubers (variety Farrukhabad) were dug at intervals of 3 days from the crop belonging to the season 1935–6 and each lot, on its arrival in the laboratory, was immediately stored at a fairly constant temperature of 12–13° C. Altogether eighteen lots were stored, the first lot being dug on 1 December 1935 when the plants were in full blossom, and the last on 21 January 1936 when the vines were beginning to dry. During the period of storage, respiration intensity, internal gaseous concentrations, composition of the atmosphere surrounding the tubers as well as values of R.Q. were determined regularly at 15-day periods. Data were also obtained with regard to the permeability of the periderm of the potato, the general method of Smith (1929) being employed.

Respiration measurements were made by the method of continuous aspiration; the carbon dioxide evolved was trapped in caustic soda and titrated against standard acid. The respiratory quotient was measured

by enclosing the material in airtight respiration chambers, and the gaseous samples removed were analysed from time to time by means of an adaptation of Haldane's gas-analysis apparatus. By employing the

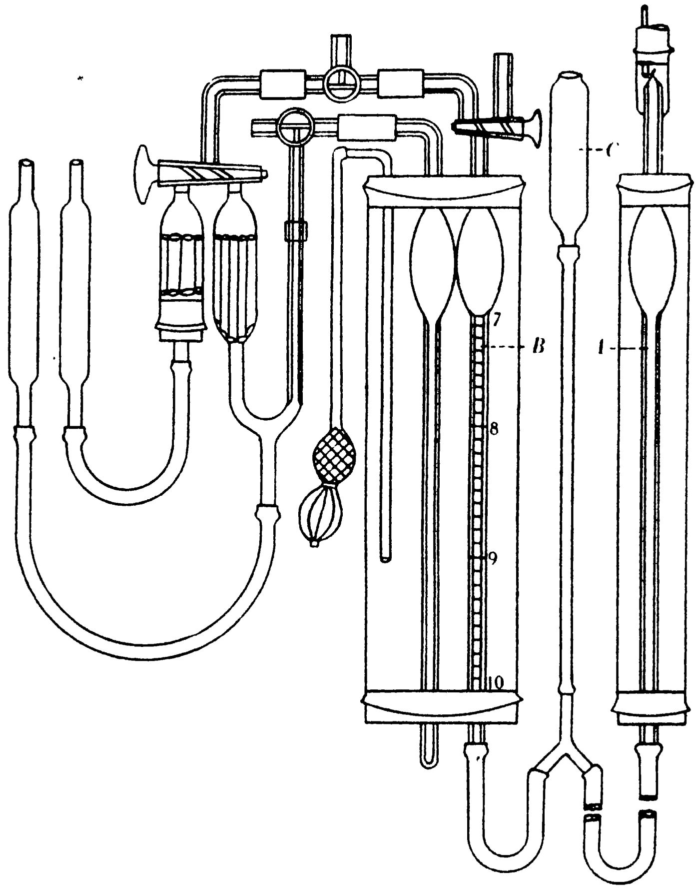

Fig. 1. Haldane gas-analysis apparatus adapted for making mechanical reductions to N.T.P. For explanation see text.

principle[1] of Lunge's gas-volumeter (Lunge & Keane, 1924; Morris, 1933) the apparatus has been improved in that the values are mechanically

[1] The principle of the apparatus is to enclose a known volume of air at such a pressure that it takes up exactly the volume which it would occupy at 0° C. and 760 mm. pressure. If the same pressure is then applied to another volume of gas this would also take up the volume which it would occupy at N.T.P.

reduced to N.T.P. without paying any attention to the readings of the thermometer and the barometer. In the modified apparatus (Fig. 1) which has been in use for about two years in this laboratory all samples of gas are measured as dry gas at N.T.P. For this purpose the "reduction" tube (*A*) is connected with the graduated pipette (*B*) and the mercury levelling bulb (*C*). Readings are taken by slightly loosening the clamp of the tube (*A*) so that it slides up or down and raising or lowering the levelling bulb till the mercury occupies the same level both in the reduction tube (*A*) and in the measuring pipette (*B*). The gas in (*B*) now occupies the volume which it would occupy when dry at N.T.P. Further details of manipulation of this apparatus have already been given by Singh & Mathur (1936*a*, *b*.).

For the determination of the composition of the interior gas, plugs of tissue from stem to eye end, 1 in. diameter, were removed from five tubers of each lot. One plug at a time was cut and pressed out of the cork borer under mercury into the gas-extraction apparatus described by Smith (1929). For determining the composition of the air surrounding the tubers, the gas samples were withdrawn with a modification of the sampler described by Singh & Mathur (1935) and analysed by means of Singh & Mathur's (1936*a*) adaptation of the Haldane gas-analysis apparatus.

Table I

Respiration intensity[1] *of potatoes of different stages of development during storage*

Date	At time of harvest	15-day periods after harvest												
		1	2	3	4	5	6	7	8	9	10	11	12	13
1. xii. 35	37·2	18·5	13·3	11·9	8·2	6·9	6·4	5·4	5·3	5·1	4·9	5·1	4·8	6·7
4. xii. 35	35·3	18·1	12·8	11·2	8·2	6·1	6·2	5·3	4·9	4·7	4·8	4·9	6·8	—
7. xii. 35	33·1	17·2	12·1	11·2	7·1	5·9	5·2	5·1	5·0	4·8	5·1	5·0	5·0	—
10. xii. 35	29·9	17·1	11·8	10·9	6·4	6·0	5·1	5·0	4·9	4·8	4·9	4·9	5·1	—
13. xii. 35	29·3	16·5	11·8	10·8	6·3	5·9	5·0	4·9	5·1	4·8	5·1	5·2	4·9	—
16. xii. 35	27·7	15·3	11·3	10·1	6·1	5·3	4·9	4·8	5·0	5·1	6·9	6·7	—	—
19. xii. 35	25·8	14·9	9·9	9·2	5·5	5·1	4·8	4·9	4·9	5·1	7·1	6·7	—	—
22. xii. 35	24·1	13·8	9·7	8·1	5·7	5·2	4·9	4·9	4·8	4·9	7·0	8·1	—	—
25. xii. 35	23·2	13·3	8·9	8·7	5·8	5·0	4·9	5·0	4·8	4·8	7·1	7·0	—	—
28. xii. 35	22·4	12·7	8·7	8·2	5·2	5·4	5·2	5·1	5·1	6·7	7·4	—	—	—
31. xii. 35	21·9	12·2	8·7	8·1	5·8	4·9	4·8	5·1	5·1	5·0	7·7	—	—	—
3. i. 36	20·5	10·0	7·9	7·3	5·7	4·8	4·9	5·0	4·9	4·9	6·8	—	—	—
6. i. 36	19·5	9·8	7·8	7·2	6·1	4·7	4·8	4·9	4·9	5·1	5·0	—	—	—
9. i. 36	18·8	9·3	7·8	7·1	6·2	4·8	4·9	5·0	4·9	5·1	—	—	—	—
12. i. 36	18·1	9·2	7·5	7·4	6·3	4·7	5·0	4·8	5·2	6·6	—	—	—	—
15. i. 36	17·0	9·1	7·1	7·0	6·2	5·0	5·0	4·9	4·9	6·7	—	—	—	—
18. i. 36	16·1	8·2	7·0	6·9	5·0	4·7	4·8	4·7	7·9	—	—	—	—	—
21. i. 36	15·5	7·9	6·2	6·1	5·5	5·6	5·4	7·3	—	—	—	—	—	—

[1] Milligrams of carbon dioxide per kg. per hour.

Results

The respiration data are presented in Table I, and a few typical records shown graphically in Fig. 2. Immediately after digging the

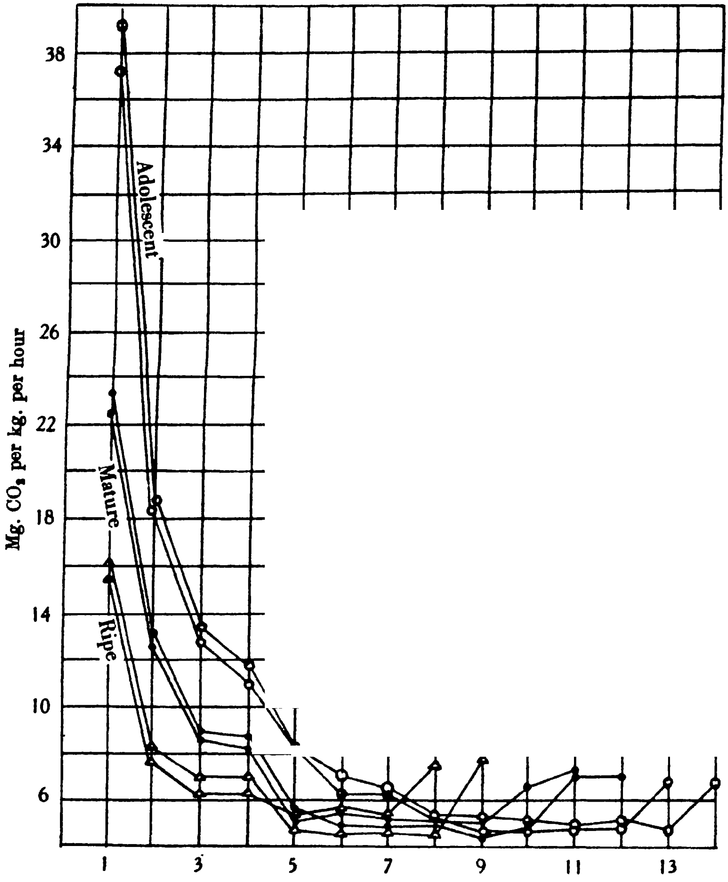

Fig. 2. Respiration rates of adolescent, mature and ripe potatoes during the storage period.

adolescent tubers exhibit a very high respiration intensity which decreases, at first rapidly and then gradually, during storage. Sooner or later the respiration intensity drops down to a level phase, the rate of respiration usually increasing again with the sprouting of the tubers.

In mature and ripe tubers the fall in respiration rate during the first fortnight is less steep and the condition characteristic of dormancy is entered into sooner. After being placed in storage the ripe potatoes are the earliest to terminate their period of dormancy.

The data regarding internal gaseous concentrations are shown graphically in Figs. 3 A and B. In the adolescent tubers the increase in the internal concentration of carbon dioxide is slow and gradually attains a more or less level phase, the concentration dropping rapidly with the commencement of sprouting. In ripe potatoes, on the other hand, the increase in the carbon dioxide concentration is rapid and is followed after the attainment of a peak value by an equally steep fall. The curves for oxygen concentration (Fig. 3 B) form more or less a mirror image of those for carbon dioxide. An increase in carbon dioxide concentration is accompanied by a corresponding decrease in that of oxygen, the value $CO_2 + O_2$ remaining practically constant.

Data concerning the composition of the atmosphere surrounding the tubers during storage are given in Figs. 3 C and D. In general, there is an accumulation of carbon dioxide in the surrounding air with increasing periods in storage. The concentration of carbon dioxide is the highest in the atmosphere surrounding the adolescent tubers, probably due to a high rate of respiration of the immature potatoes. The percentage of oxygen in the atmosphere surrounding the tubers progressively decreases with an increase in the length of the storage period.

Table II records the composition of samples of air withdrawn from top, middle and bottom layers. Mature potatoes were placed in bins 4 ft. long and 3 ft. in diameter and were piled to a depth of 3½ ft. in three layers, each layer 14 in. deep and separated from the adjoining one by ½ in. mesh wire netting. The data show a slightly higher percentage of

Table II

Effect of depth of piling potatoes on the composition of the atmosphere surrounding the tubers

Layer	At time of harvest	15-day periods after harvest												
		1	2	3	4	5	6	7	8	9	10	11	12	13
						Carbon dioxide								
Top	0·05	0·06	0·07	0·09	0·09	0·11	0·11	0·13	0·13	0·14	0·14	0·15	—	—
Middle	0·05	0·07	0·08	0·09	0·11	0·12	0·12	0·13	0·14	0·17	0·19	0·21	—	—
Bottom	0·05	0·09	0·11	0·12	0·14	0·21	0·22	0·25	0·27	0·29	0·31	0·31	—	—
						Oxygen								
Top	20·8	20·7	20·5	20·2	19·8	19·7	19·6	19·6	19·5	19·4	19·2	19·1	—	—
Middle	20·8	20·0	19·9	19·8	19·8	19·7	19·6	19·5	19·4	19·1	18·8	18·7	—	—
Bottom	20·8	19·9	19·7	19·6	19·4	19·2	18·9	18·7	18·6	18·6	18·6	18·6	—	—

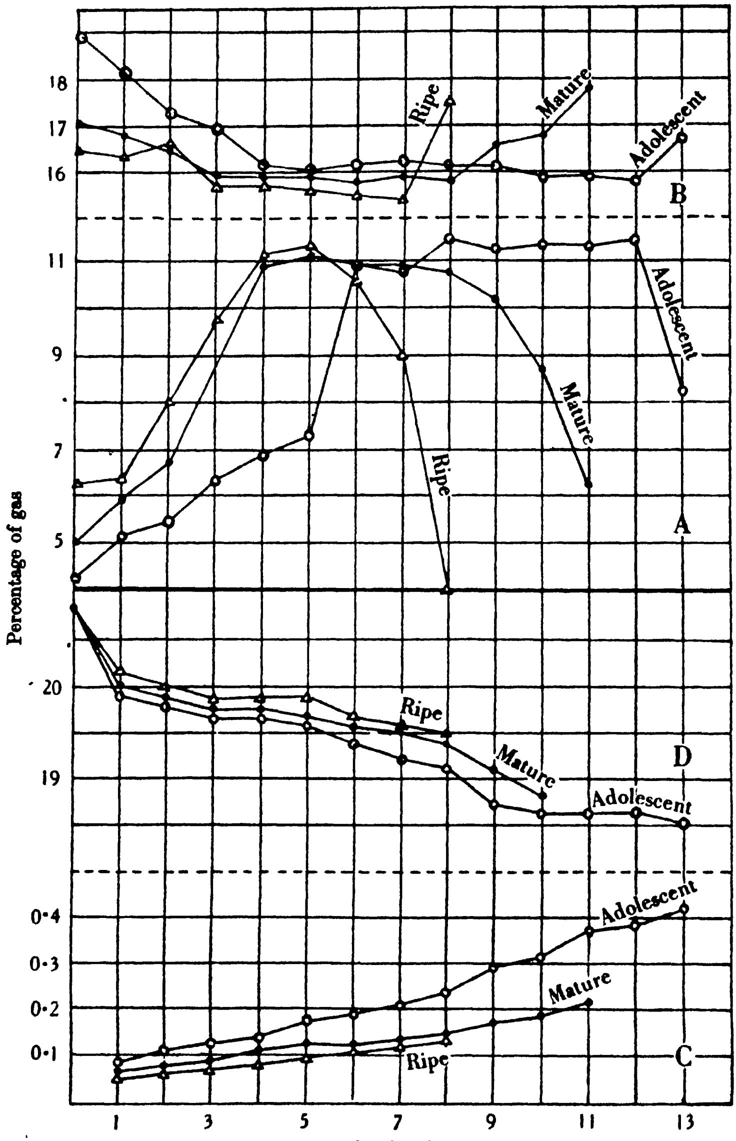

Fig. 3. Composition of the internal and external atmospheres of tubers during storage. A, internal carbon dioxide; B, internal oxygen; C, external carbon dioxide and D, external oxygen.

carbon dioxide in the bottom layer than in the middle and top ones. This higher percentage in the bottom layer may have been due to any or all of the following factors: greater respiratory activity, inability of gases to escape, and accumulation from layers above due to the density of the gas.

The values of R.Q. during storage are presented in Table III. In adolescent tubers the R.Q. starts with a value of about unity, drops down to 0·90 during the period of dormancy and rises again to 1·01 at the time of sprouting. A similar course of events applies to mature and ripe potatoes. A significant point in this connexion is the negative correlation between R.Q. and the internal carbon dioxide concentration during the periods of dormancy and sprouting (Fig. 3 A and Table III), higher values of R.Q. being invariably associated with lower carbon dioxide concentrations. It appears probable that with the onset of dormancy more and more carbon dixoide accumulates within the tissues of the tuber on account of a decrease in the superficial evolution of the gas.

Table III

Values of R.Q. of potatoes of different developmental stages during storage

Developmental stage	At time of harvest	15-day periods after harvest												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Adolescent	0·98	1·00	0·99	0·99	0·98	0·98	0·97	0·91	0·90	0·90	0·91	0·90	0·91	1·01
Mature	0·99	1·02	1·00	1·00	0·91	0·90	0·91	0·91	0·92	0·92	0·99	1·03	—	—
Ripe	0·99	0·99	0·98	0·95	0·94	0·90	0·92	0·92	0·99	—	—	—	—	—

Data concerning the permeability of the periderm of the potato to gas during storage (Table IV) indicate that the permeability of the superficial tissues decreases considerably during the dormancy of the tubers, a slight increase being discernible during sprouting. This explains the accumulation of carbon dioxide and the depletion of oxygen in the tubers during dormancy.

Table IV

Permeability of the periderm of the potato to gas during storage

Developmental stage	At time of harvest	15-day periods after harvest												
		1	2	3	4	5	6	7	8	9	10	11	12	13
		Gas extracted from eight potatoes, ml.[1]												
Mature	5·6	5·0	5·0	4·8	4·3	4·1	3·6	3·1	3·1	3·2	4·5	4·6	—	—

[1] Average of ten determinations.

Discussion

In the case of storage organs, the problem of the relation between the rate of respiration on the one hand, and the internal and external concentrations of carbon dioxide on the other, is of considerable physiological significance. Its theoretical importance lies in the fact that the rate of respiration—the amount of carbon dioxide evolved superficially per unit weight—is influenced considerably by the composition of the atmosphere within the plant organs, which is in equilibrium with the amount of gas dissolved in the tissue sap as well as by the concentration of carbon dioxide in the surrounding atmosphere, which forms the immediate environment of the tubers. Since the permeability to gases of the periderm of the potato varies during storage of tubers it might be inferred that the superficial evolution of carbon dioxide will not bear a fixed ratio to its production during metabolism, as a result of which both the composition of the internal atmosphere of tubers as well as the rate of respiration will fluctuate within certain limits. The rate of carbon dioxide evolution under various circumstances will be conditioned, therefore, among other factors by (1) the concentration of carbon dioxide inside the tuber, (2) the composition of the atmosphere surrounding the tubers, and (3) the permeability of periderm to diffusion of gases.

In potato storage studies, therefore, considerable attention should be given to the development of the periderm and the various factors that influence its permeability to gases.

Summary

1. Adolescent, mature and ripe potatoes continue to be distinguished by their respiratory behaviour throughout the period of storage.

2. When potatoes are placed in storage there is a progressive increase in the concentration of internal carbon dioxide until the termination of the period of dormancy, the percentage of this gas falling rapidly with the commencement of sprouting.

3. Data concerning the composition of the atmosphere surrounding the tubers show that, in general, there is an accumulation of carbon dioxide in the surrounding air with increasing periods in storage.

4. There is a negative correlation between R.Q. and the percentage internal carbon dioxide during the stages of dormancy and sprouting.

5. Records concerning the composition of samples of air withdrawn from top, middle and bottom layers of potatoes show a slightly higher

percentage of carbon dioxide in the bottom layer than in the middle and top ones.

6. Data concerning the permeability of the periderm of the potato to gas during storage indicate that the permeability of the superficial tissues decreases considerably during the dormancy of tubers.

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FUNGI CAUSING ROTS OF APPLE FRUITS IN STORAGE IN NORTHERN IRELAND

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CONTENTS

I. INTRODUCTION

THE investigation of the fungi responsible for the decay of apple fruits under ordinary storage conditions was undertaken in order to obtain some knowledge of the parasites responsible for storage losses in Northern Ireland, and to bring the position of the Province into line with that of other apple-growing countries where, in practically every case, information on this subject is available.

Up to the time of the commencement of the work little attempt had been made to study the storage rots of Irish-grown apples, so that few published records as to the fungi responsible for such losses are in existence. Lafferty & Pethybridge (1922) reported the occurrence of *Phytophthora Syringae* Klebahn as causing a rot of Irish apples. Muskett *et al.* (1931) reported the occurrence of *Phytophthora cactorum* (Leb. & Cohn) Schroet. and *Tricothecium roseum* Link on apples in Northern Ireland, and of *Penicillium expansum* Thom (Muskett *et al.* 1934), but pathogenicity was not tested. During the course of the present investigation, *Corticium centrifugum* (Lev.) Bres. was found to be the cause of a rot observed on a few apples grown and stored in Co. Armagh. The occurrence of this fungus was reported shortly after its identification (Colhoun & Muskett, 1935).

II. EXPERIMENTAL METHODS

During the years 1934 and 1935 visits were made at frequent intervals to a number of apple stores at orchards in the counties of Armagh, Tyrone and Antrim, this area comprising the most important apple-growing districts in Northern Ireland. From these stores typical samples

of the various rots occurring were selected, and isolations made from all rots present on these specimens. When pure cultures of the fungi isolated had been obtained they were tested for pathogenicity by carrying out artificial inoculations on apples of the variety Bramley's Seedling, the method of inoculation adopted being that described by Granger & Horne (1924). The inoculated apples were placed in moist chambers and held at laboratory temperatures. Where a rot was produced in the inoculated apples, a reisolation of the parasite was made. Organisms which did not produce rots in apples artificially inoculated at the beginning of the storage season were tested for pathogenicity at intervals until the end of the season.

III. THE FUNGI CONCERNED

The total number of species and strains isolated numbered over forty, and of these the following have been proved pathogenic to Bramley's Seedling apples:

Phytophthora Syringae Kleb. (strains 1, 2 and 3).
Mucor racemosus Fres.
M. piriformis Fischer.
Penicillium expansum Thom.
Botrytis cinerea Pers. (strains 1, 2 and 3).
Corticium centrifugum (Lev.) Bres.
Phoma mali Schulz & Sacc. (strains 1 and 2).
Phomopsis mali Roberts.
Cytosporella mali Brun.
Gloeosporium fructigenum Berk.
Colletotrichum gloeosporioides Penz.
Trichothecium roseum Link.
Fusarium lateritium Nees var. *fructigenum* (Fr.) Wr.
Fusarium avenaceum (Fr.) Sacc.
Sporotrichum sp.
Verticillium sp.

In addition to these species a number of other organisms have been proved pathogenic but have remained sterile on the apple fruit and on all media employed, and consequently have not been identified.

Practically all the organisms isolated were found producing rots on apples of the variety Bramley's Seedling which is the main variety grown in Northern Ireland. In the following account of the fungi and the rots they produce the variety of apple from which the organisms were originally isolated was Bramley's Seedling unless otherwise stated.

Phytophthora Syringae Kleb.

There appear to be only two species of *Phytophthora* responsible for disease of apple fruits in nature, these being *P. Syringae* Klebahn and *P. cactorum* (Leb. & Cohn) Schroet., of which the latter appears to have the wider distribution as regards its occurrence on apples, having been recorded in England (Wormald, 1919), Northern Ireland (Muskett *et al.* 1931), Switzerland (Osterwalder, 1906) and the United States (Clinton, 1920; Haskell & Wood, 1922; Rose & Lindegreen, 1925; Whetzel & Rosenbaum, 1916; U.S. Dep. Agric. 1918). *P. Syringae* has been recorded as being prevalent in the south-west of England (Ogilvie, 1932), and in the Irish Free State (Lafferty & Pethybridge, 1922).

During the present investigation three distinct strains of *P. Syringae* were isolated and studied, these being obtained from apples in the early part of the storage season when considerable damage is often caused. All three strains are capable of producing rots of healthy apples. The rots produced are mid-brown in colour, fairly firm to the touch in the early stages of the disease, but become softer later. The diseased internal tissue is light brown in colour with the vascular strands showing up as darker brown threads. No mycelium was observed on the surface of the apple.

The strains of *P. Syringae* studied produced sporangia when grown on 2% oat agar or on 2% malt extract agar, although the production of sporangia was more frequent in some cases. The production of sexual organs on either of these media was not observed, but when small pieces of diseased tissue from inoculated apples were incubated in sterile water for a number of days, oogonia and antheridia, which were predominantly paragynous, were produced.

The technique described by Leonian (1934) was employed in assigning the strains to the species *P. Syringae*. When plates of Leonian's medium were inoculated with any of the three strains and incubated at 27° C. no growth resulted. Dishes of sterile pea broth were inoculated with hyphae of each strain and incubated at 20° C. for 3 days. The colonies formed were then thoroughly washed in sterile distilled water, and transferred to Petri dishes which contained enough sterile distilled water to cover their bottoms. After 6 days' incubation at 20° C. it was found that all three strains produced sporangia freely, but sexual organs did not develop. The sporangia when mature were rounded or slightly flattened at the apex and without a definite papilla, thus resembling those of the organism described by Lafferty & Pethybridge (1922) as being *P. Syringae*.

Many workers have reported that infection of apples with *Phytophthora* takes place from the soil so that windfalls are chiefly liable to be attacked. A considerable number of apples were collected in October 1935 on a damp day, from the ground under trees in a grass orchard, and allowed to dry in the laboratory. They were then carefully examined and any showing signs of infection with disease were discarded. The remainder were stored in a cool room for 18 days and at the end of this period at least 25 % of them showed infection with *Phytophthora*. A number of cases have been reported in Northern Ireland where apples on pulling had been allowed to lie on the ground in the orchard for a few days before storing, and after two or three weeks in store showed signs of *Phytophthora* rot.

Lafferty & Pethybridge (1922), in reporting the occurrence of *P. Syringae* in Ireland, did not consider that it was likely to become a serious menace to Irish fruit growers, but the present study shows that the disease may, under certain conditions, be of some importance in Northern Ireland.

During this investigation an organism isolated from pears, and which is believed to be *P. cactorum*, was studied. This organism is also pathogenic to apple fruits.

Mucor racemosus Fres. and *M. piriformis* Fischer

The species of *Mucor* occurring on apples in Europe may be referred to *M. racemosus* Fres., *M. mucedo* Linn., and *M. piriformis* Fischer. All three species have recently been reported as causing rots in Bohemia (Baudys, 1931). In America *M. piriformis* has been recorded (Heald & Ruehle, 1931), but it is stated to be an infrequent cause of decay.

Two species of *Mucor* were isolated during the study, and these considered together rank among the four most important diseases of stored apples occurring in Northern Ireland. They proved to be *M. piriformis* and *M. racemosus*, the latter being the more common. Both are more prevalent during the early part of the storage season. The rots produced by the two species are indistinguishable, the lesions are light brown in colour, soft and watery, and the rot progresses very rapidly. The diseased internal tissue is light brown in colour and very soft. In well-rotted fruits sporangiophores appear at breaks in the skin or emerge through lenticels. When well-rotted apples are kept in an airtight chamber for some days a characteristic alcoholic odour is noticed.

Penicillium expansum Thom.

A considerable number of species of *Penicillium* are responsible for loss in stored apples, but of these *P. expansum* is by far the commonest. During the present survey *P. expansum* was the only species of the genus isolated. It appears to be widespread in the province, being the most serious source of damage to stored fruit. The rot produced is light brown in colour, soft and spreads very rapidly. The diseased internal tissue is very watery and pale in colour with the vascular strands showing up as darker brown threads. Spores are produced in coremia on the surface of the apple quite early in the development of the rot and in great abundance. In the early stages of its development the sporulating growth is white in colour, but as the spores mature it becomes blue-green. Well-rotted apples often exhibit a musty odour. *P. expansum* usually gains entrance to the fruit through wounds but is also capable of penetrating lenticels (Horne & Eweis, 1934).

Botrytis cinerea Pers.

B. cinerea is the most common species of this genus occurring on the apple fruit, and it has been reported from most apple-growing countries. A number of distinct strains were isolated during this work, and while all these strains are capable of causing rots they differ in some respects as regards cultural characteristics, chiefly in their capacity to produce conidia and sclerotia.

The rots produced by the various strains are indistinguishable, being light brown in colour and firm to the touch but becoming softer later. A few weeks after inoculations are made whitish tufts of fungal growth appear on the surface of the apples. Conidiophores bearing conidia have been frequently observed on apples at points where the skin was broken.

Corticium centrifugum (Lev.) Bres.

This species has been recorded in Europe on dead twigs, etc. (Burt, 1926), but has not been observed to cause disease of home grown apples in Britain until isolated during the present survey (Colhoun & Muskett, 1935). In 1930 the disease was recorded in England on apples imported from Canada (Rome, 1931). It has been studied in America, where it is widespread, by Eustace (1903), Butler (1927, 1930), and Heald & Ruehle (1931). The earlier workers considered the disease to be due to *Hypochnus* sp., but Butler (1930) assigned the causal organism to the genus *Corticium*.

The lesions resulting from natural infection may or may not centre

around scab spots, but when a scab spot occurs in the centre of a lesion there is a marked resemblance to the eye of a fish, the black patch in the centre caused by the scab fungus (*Venturia inaequalis*) being surrounded by tan-coloured tissue with a ring of dark brown around the outside. Lesions often coalesce to form a large diseased patch. The diseased area is usually quite firm and slightly sunken. When diseased apples are kept in a moist chamber the fine white mycelium of the fungus forms a cobweb-like mat over the surface of the fruit. The production of spores on the apple fruit was not observed. The diseased internal tissue is light brown in colour, dry and stringy, giving the whole a somewhat spongy consistency.

The fungus grows readily on most of the common media, and on malt agar, which was the medium chiefly used in the work, basidiospores are produced in abundance, and clamp connexions develop on the mycelium. These characters, together with a sweet, penetrating, aromatic odour emitted by cultures, assist in the identification of the fungus.

In Northern Ireland the disease has only been found in one orchard, and in that case only a few apples were affected. The disease is essentially a late storage rot. Butler (1930) has suggested that infection of the fruit takes place in the orchard, the fungus establishing itself in tissues of the mature fruit which are dead or at a low ebb of life, such as stems, calyces or lenticels. The disease then develops later in storage. Both Butler (1930) and Eustace (1903) considered that the fungus could not penetrate sound epidermis.

Phoma mali Schulz & Sacc.

Apart from the record of Massee (1915) who, when working on blister disease of fruit trees in England, decided that *Coniothecium chomatosporum* Corda, *Phoma mali* and *Diaporthe ambigua* Nits. are different stages of one organism, which causes a blister disease of young shoots and also attacks the fruit, this species has been proved by Lewis (1909) to be capable of causing a rot of apples in America.

In the present study two strains of *P. mali*[1] have been found causing rots of apples. The rot produced on artificial inoculation by strain 1 is light brown in colour and firm, but becomes softer as the disease progresses. The rot spreads rapidly in ripe apples, and about 4–5 weeks from the time of inoculation, pycnidia appear beneath the skin of the apple. A few weeks later the pycnidia burst through the epidermis and

[1] Both strains were kindly identified by Dr Joh. Westerdijk, Centraalbureau voor Schimmelcultures, Baarn.

masses of small oval spores are exuded from each. The spores which measure $7{\cdot}1 \times 3\,\mu$ are slightly larger than those reported by Lewis (1909). Strain 2 produces a rot which is dark brown in colour and very firm to the touch, but within 10 days from the time of inoculation, a large number of pycnidia appear just under the skin of the diseased area and finally the whole surface becomes black. About a month after inoculation the pycnidia burst through the cuticle and the spores emerge. These spores measure $7{\cdot}1 \times 2{\cdot}9\,\mu$.

The behaviour of the strains has been studied on a number of different media. Strain 1 has never produced spores in culture on any of the media employed, although sterile black bodies have developed usually on 2 % oat agar or on Crabill's medium. Strain 1 produced slight blackening of the medium when grown on malt extract or potato mush agar, but no blackening has been observed on oat or prune agar or on Crabill's medium. Strain 2 produced abundant unilocular pycnidia and spores on most media, where it also caused very distinct blackening of the medium.

P. mali appears to be of considerable importance in bringing about loss of stored apples in Northern Ireland, especially towards the close of the storage season. From observations made in the stores it appears that the fungus gains entrance chiefly through the stalk end of the fruit.

Phomopsis mali Roberts

This organism, which is regarded as the pycnidial stage of *Diaporthe perniciosa* Marchal, has been recorded on apple fruits in England (Kidd & Beaumont, 1924) and America (Roberts, 1912). Kidd & Beaumont (1924) state that the fungus causes much loss late in the storage season. This has been confirmed in Northern Ireland where, in a number of stores, *Phomopsis mali* was the dominant species causing rots from Christmas onwards.

The rots produced are mid-brown in colour, fairly firm to the touch and spread rapidly. In well-developed rots small pycnidia appear just below the skin and are densely crowded all over the fruit. Eventually these pycnidia, which are unilocular, burst through the skin and liberate small masses of spores, both A and B spores being produced.

In old cultures a few large black stromata are produced. The pycnidia are unilocular and produce masses of A and B spores.

Cytosporella mali Brun[1]

This species was recorded in 1924 for the first time on apple fruits by Kidd & Beaumont (1924) in England, where it was isolated on one occasion.

In Northern Ireland it was isolated on three occasions during the survey. The rots from which isolations were made were centred around injuries or the stalk end of the fruit. The rot produced by the fungus is dark brown in colour, becoming darker towards the centre of the diseased area. Pycnidia are produced abundantly in culture but their production on diseased fruit has not been observed.

Gloeosporium fructigenum Berk.[1]

This species appears to have a wide distribution and has been reported from most apple-growing countries. In England it was isolated on two occasions by Kidd & Beaumont (1924). In Northern Ireland the fungus does not appear to be of much importance in causing rots of apples, since it has only been isolated on one occasion from a few apples towards the end of the storage season. The rot produced is dark brown in colour and slightly soft.

Colletotrichum gloeosporioides Penz.

This fungus, which is known to be a common parasite of oranges, has been previously recorded in England on apples by Kidd & Beaumont (1924), who found it occurring on one occasion.

During the present work *C. gloeosporioides* was isolated on one occasion from a few apples of the variety Golden Spire, and it has been observed in another orchard on apples of the variety Bramley's Seedling. The rot produced on Bramley's is dark brown in colour and rather soft. As the rot spreads, the pinkish coloured acervuli appear in great abundance on the surface of the fruit.

In culture on malt agar the acervuli appear in abundance, but with frequent subculturing the power to produce spores decreases. The acervuli produced in culture and on the apple contain numerous dark coloured setae which are most easily seen in young pustules.

Trichothecium roseum Link

This is a widely distributed species and is stated on some occasions to cause considerable damage following scab. The organism was isolated in this survey on a large number of occasions from small spots centring

[1] Thanks are due to Dr Joh. Westerdijk for identification of this species.

around scab lesions. In the store a whitish fungal growth appears over the surface of the scab spot, and later the tissue attacked by the fungus becomes sunken, forming a narrow brown coloured band around the scab lesion.

Fusarium lateritium Nees var. *fructigenum*[1] (Fr.) Wr.

As far as stored apples in Northern Ireland are concerned *F. lateritium* var. *fructigenum* does not appear to cause much loss since it was isolated once only. The strain isolated was but feebly parasitic even towards the end of the season, although its powers of bringing about attack increase as the season advances. The rots produced are dark brown and slightly sunken, but fructifications of the fungus are not produced on infected fruits. This organism was also successfully isolated from buds of the variety Grenadier from Co. Armagh, where it is responsible for causing a bud rot.

Fusarium avenaceum (Fr.) Sacc.[1]

In recording the occurrence of this species on apples in England, Kidd & Beaumont (1924) state that it is not of great importance in storage. During this study *F. avenaceum* was first isolated in the autumn 1934 from a small shrivelled apple remaining on the tree. Later it was isolated on four occasions from fruit stored at different orchards, but in no case was the damage severe.

The rot produced is dark brown in colour, fairly soft, and slow in spreading. In cases where the disease is well advanced the diseased portion of the flesh becomes sunken, and a weft of hyphae covers a large portion of the surface of the diseased tissue. As far as could be ascertained from observations in the store infection usually takes place through the stalk end of the fruit.

Sporotrichum sp.

This organism was isolated on a few occasions from small dark brown lesions towards the end of the storage season. Inoculation experiments show that it is feebly parasitic and will attack Bramley's Seedling apples only when the storage season is well advanced. It has not yet been possible to assign the organism isolated to a definite species.

Verticillium sp.

This organism was isolated on one occasion during the work and was found to be feebly parasitic even at a very late period in the storage season. It has not yet been definitely identified.

[1] Cultures of both species of *Fusarium* were kindly identified by Dr Wollenweber.

IV. DISCUSSION

During this investigation the variety Bramley's Seedling received most consideration, since this is the variety most largely grown on a commercial scale in Northern Ireland. Being a sour variety, and as, in this work, little attention was paid to fungi causing lenticel spotting at the close of the storage season, the number of organisms studied has been limited.

It was observed during the course of the work that a large proportion of the rots present on apples in storage in Northern Ireland centred around wounds or the stalk or calyx ends of the fruit. The rots centring around lenticels were comparatively few in number until the storage season was far advanced.

Horne (1931) has shown that the primary infection of apples contracting disease during storage can be traced to the orchard from which such apples were obtained, for it was found that the fungi present in the tissues of apples contracting disease were also present on the fruit before it was detached from the tree. Carter (1935), in her study of the fungi in air over orchards, produced evidence in support of the suggestion that diseases of apples in storage are due mainly to fungal infection from the atmosphere before the picking of the fruit. Further, Ogilvie (1935) has shown that many of the fungi capable of attacking apple fruits may also lead a parasitic or saprophytic existence on other parts of the apple tree, chiefly on the limbs.

It would therefore appear that much may be done to improve the keeping quality of the fruit by paying strict attention to hygienic conditions within the orchard. The exercise of greater care in general orchard management should do much to reduce the number of spores present in the atmosphere. The reduction of fruit losses due to fungal decay will also be achieved by carrying out regularly an efficient spraying programme for the control of diseases and pests; by the more careful handling of the fruit during and after picking; and by taking care not to leave the fruit lying on the ground in the orchard after picking.

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COMPLEX FUNGAL ROTTING OF PEA SEEDS

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(With Plates I and II)

CONTENTS

Introduction

Poor germination of peas of both garden and field varieties sometimes reaches such proportions as to be a limiting factor in pea growing. The causes of these "poor plants" in England have never been definitely determined. Several fungi are important in causing diseases of the stems or other parts of peas, and these may be placed in three groups distinguishable by the symptoms produced.

Group I

Fungi causing primarily spotting of the pods, and later of the cotyledons of the peas, which leads eventually to a poor stand of plants and a certain amount of distortion of the stems with more or less foot-rot.

Mycosphaerella pinodes Berk. & Blox. According to Ogilvie & Mulligan (1932) this fungus can cause severe foot-rot in England, in addition to blight of the pods and leaves as described by Jones (1927).

Ascochyta pisi Lib. Ogilvie & Mulligan (1932) found that this fungus, primarily a leaf-spotting and pod-spotting organism, causes little foot-rot, producing only brown sunken lesions on the underground part of the stem.

Ascochyta pinodella L. K. Jones. Jones (1927), who names this species, found it to cause foot-rot in addition to spotting of the pods and leaves.

Group II

Fungi causing primarily wilting, accompanied occasionally by slight foot or root-rot.

Fusarium orthoceras App. & Wr. var. *pisi* Linford. Apart from typical wilt symptoms, the plants infected by this fungus show a limited superficial browning of rootlets but are otherwise white and clean externally. Marked vascular discoloration occurs (Linford, 1928).

Fusarium oxysporum Schl. f. 8, Snyder. Causal organism of an important wilt, called "near-wilt" of peas in U.S.A. Snyder & Walker (1935) found that, after 8 weeks' growth in soil heavily inoculated with the fungus, many of the small roots of pea plants were bronzed and decayed, but the underground macroscopic symptoms were on the whole slight when compared with the severe wilting which occurred.

Fusarium viticola Thuem. on wilted pea, Sweden (Togashi, 1928–9).

Fusarium culmorum (W. G. Smith) on wilted pea, Sweden (*ibid.*).

Fusarium dimerum Penzig, on wilted pea, Sweden (*ibid.*).

Fusarium vasinfectum Atk. var. *pisi* van Hall, causing St John's wilt in Holland (*ibid.*).

Fusarium conglutinans Wr. on wilted pea in U.S.A. (*ibid.*).

Group III

Fungi causing primarily root and foot-rot.

Aphanomyces euteiches. According to Jones & Drechsler (1925) this fungus enters only the cortex of the roots and base of the stem where it softens and decays the tissue, exposing the vascular system to decay by other organisms. According to Jones & Linford (1925) in their survey of Wisconsin pea diseases "during the 1924 survey the root-rot of peas caused by *Aphanomyces* was found to be far more important than all the other parasitic diseases combined, causing losses amounting to approx. 8 % of the yield of the total acreage inspected".

Rhizoctonia solani. Miss Gilchrist (1926) illustrates this fungus causing severe foot-rot, the cotyledons being practically rotted away, and considerably more severely infected than with *Ascochyta pinodella.* Jones & Linford (1925) state that this fungus causes greatest injury when invading very young tissues, though it may attack any underground portion of the plant. They further state "It may enter germinating seeds,

killing the embryo or destroying the cotyledons, removing the food reserve of the developing seedlings. It may attack seedlings before emergence from the soil, injuring or completely destroying the growing points of roots and stem."

Fusarium solani (Mart.) var. *martii* (Appel & Wr. subspecies) Wr. f. 2, Snyder. This fungus, originally named by Jones (1923) *Fusarium martii* var. *pisi*, and renamed by Wollenweber and Snyder, has come to be recognized as one of the most important and widespread of the pea root-rotting fungi. Various strains of this fungus, and single spore strains in various conditions of culture, show wide variability (Snyder, 1934). Hence the original contention of Jones, that this fungus "must be distinguished on morphological characteristics which are constant", has had to be modified, and Snyder uses pathogenicity as a major characteristic in distinguishing this fungus from closely related forms and subspecies of *F. solani*.

This fungus causes a severe rotting of the stem and roots, discolouring them reddish brown to almost black. It may cause rotting of the cotyledons. It may, in addition, cause a wilt, as indicated by Jones (1923). According to Johanna Went (1934) it is a chief cause of St John's wilt in Holland. According to F. R. Jones it is the only species of *Fusarium* causing serious foot-rot in the U.S.A., and Ogilvie *et al.* (1934) hold the same views for England.

Other species of *Fusarium* which have been mentioned in the literature are the following:

F. arthrosporioides Sherb.
F. sporotrichioides Sherb.
F. anguioides Sherb. (Togashi, 1928–9).
F. avenaceum (Fr.) Sacc. (*ibid.*).
F. herbarum (Corda) Fr. (*ibid.*).
F. herbarum var. *gibberelloides* Wr. (*ibid.*).
F. martii Appel & Wr. (*ibid.*).
F. martii var. *minus* Sherb. (*ibid.*).
F. falcatum Appel & Wr. (*ibid.*).
F. redolans Wr. (*ibid.*).
F. blasticola Rostrup (*ibid.*).
F. scirpi Lamb. & Fautr. var. *acuminatum* (Ell. & Everh) (Wollenweber & Reinking, 1935).
F. equiseti (Corda) Sacc. var. *bullatum* (Sherb.) Wr. (*ibid.*).
F. merismoides Corda (*ibid.*).
F. equiseti (Corda) Sacc. (*ibid.*).

The descriptions given of the various diseases by the different authors rarely leave any doubt as to the group in which any of the causal organisms may be placed. Throughout the literature, however, stress is laid upon one of the symptoms, pod spotting (with subsequent lesions developed on the young peas), wilting or root-rotting. In spite of the importance of securing a good "stand" of this crop, the rotting of seed in the soil is frequently overlooked.

Substantial increases in stand resulting from dressing pea seed have been known for some time, and the writer had the opportunity to investigate cases where very spectacular improvements were obtained from dressing seed with certain experimental dressings. The experiments were conducted in five widely separated localities. Recently a paper has been published by Brett *et al.* (1937) in which similar experiments with mercurial seed dressings gave large increases.

Early experiences showed that it was no simple matter to isolate parasitic fungi from rotting seed; usually an abundance of moulds and bacteria was the reward of large numbers of isolation plates. As a supply of the original seed had been secured from each of the five centres, this served to supply the necessary information on diseases carried on the seed. Naturally, *Ascochyta* and *Mycosphaerella* were sought as the disease organisms. In addition, stems and roots of almost mature plants were secured from each centre; they showed an abundance of superficial rotting and served as an indication of root and stem parasites present. Finally, a number of stock cultures of widely separated fungi, mostly obtained from plants other than peas, completed the source of biological material.

Experimental methods

(a) *Isolation of cultures*

A simple method of surface sterilizing tissue was found very satisfactory in isolating *Ascochyta pisi*, many species of *Fusarium* and other fungi from stems, roots and seeds of peas. It is similar to that described by Davies (1935) for isolating *Ophiobolus graminis* from wheat stems. The tissue was soaked for 10–60 min. in distilled water, dipped for 2–3 min. in 1 % silver nitrate solution, washed in concentrated sodium chloride solution, and plated on potato-dextrose agar.

(b) *Media*

The media used for identification of fungi were those of Wollenweber *et al.* (1925). Ridgway's colour chart (1912) is used throughout for colour descriptions. In making certain spore measurements, assistance was given by Mr R. P. Libbey of Reading University.

(c) *Testing for pathogenicity*

The fungi were grown for 3 weeks in a sterilized mixture of 190 g. sand, 10 g. cornmeal and 80 c.c. water in flasks. This was mixed with three times the quantity (air-dry weight) of sterilized or unsterilized sandy loam. 100 g. were placed in a tumbler, ten pea seeds were sown, and the seeds were covered with more infested soil. The moisture was brought to 50 % saturation. The seedlings were grown at a convenient temperature. In one instance, boxes containing twenty-five seeds were used.

Fungi isolated

(a) *Fungi isolated from seed*

Table I indicates the types of fungi isolated from pea-seed samples taken from that used in the five field trials referred to above. In the table are given the variety of pea sown in each locality together with the average percentage increase resulting from the use of the five experimental dressings. These figures are a conservative indication of the benefit of seed dressings, inasmuch as they include one dressing distinctly inferior to the others in preventing rotting. Thirty seeds of each variety were sterilized with silver nitrate and plated on potato-dextrose agar. In separate columns are listed the number of peas showing complete freedom from contamination, the number showing species of either *Penicillium* or *Aspergillus*, and those showing other fungi, including an occasional isolate of a species of *Fusarium.*

Table I

Fungi isolated from seed used in field experiments

Variety	Locality	Increased field germination %	Peas free from contamination No. per 30 seeds	Peas showing *Penicillium* or *Aspergillus* only No. per 30 seeds	Peas showing other fungi No. per 30 seeds
Queen	Peterborough	15·8	29	0	1
Thomas Laxton	Rochester	58·6	16	10	4
Market Gardener	Rochford	50·9	2	22	6
Foremost	Evesham	3·2	22	7	1
Thomas Laxton	Rainham, Essex	16·7	22	1	7

None of the peas yielded parasitic fungi in appreciable quantities. The *Penicillium* and *Aspergillus* species were found to be non-parasitic, and among the "other fungi" neither *Ascochyta* nor *Mycosphaerella* was present. This was in spite of the fact that the method used for isolating has proved satisfactory for isolating *Ascochyta* from seeds, as will be seen later. It is evident that if the cause of rotting in the field was seed-borne

fungi, then they were very superficially borne and were destroyed by surface sterilization.

Seeds of four commercial varieties of peas well known to suffer from poor germination were then examined. Seeds of each variety were sown in unsterilized field soil in glazed earthenware pots, and others were sown in similar soil which had been sterilized in an autoclave at 20 lb. pressure for 2½ hr. After 14 days' growth at 50° F. the plants were lifted, and one cotyledon from each plant was surface sterilized with silver nitrate. The fungi isolated from the cotyledons of each variety are listed in Table II.

Table II

Isolation from cotyledons of seedlings of varieties Union Jack, Thomas Laxton, Early Bird, and Little Marvel

Variety	Soil	No. of plants examined	*Penicillium* only	*Fusarium* sect. *Elegans*	*Fusarium* sect. *Roseum*	*Fusarium culmorum*	*Botrytis cinerea*
Union Jack	Sterilized	22	18	4	0	0	0
	Unsterilized	13	0	11	1	1	0
Thomas Laxton	Sterilized	19	16	3	0	0	0
	Unsterilized	20	1	19	0	0	0
Early Bird	Sterilized	18	12	4	0	2	0
	Unsterilized	18	4	13	0	1	0
Little Marvel	Sterilized	13	10	2	0	0	1
	Unsterilized	22	10	11	0	1	0
Little Marvel	Sterilized	20	10	5	0	4	1
	Unsterilized	17	0	12	0	5	0

It will be noted that there was a strong tendency for *Fusarium* of section *Elegans* to occur on peas from unsterilized soil, while in the sterilized soil *Penicillium* was far more frequent. It seems not improbable that both fungi, or groups of fungi, were carried on the seed, one being favoured by sterilized soil and the other by unsterilized soil. It would also appear that *Fusarium culmorum* and *Botrytis cinerea* were carried on the seed. Several of the isolates tested later were found to be highly pathogenic, but they were not species of *Penicillium* or *Fusarium* section *Elegans*.

(*b*) *Fungi isolated from foot-rot material*

At the time when peas were picked from the five experimental centres referred to above, a large amount of foot-rot was present in all cases; in fact, it was difficult to find plants completely free from rotting. Plants were saved from each centre, no special effort being made to secure diseased material. At the same time pods showing spotting or rotting were collected from Evesham and Rochester. No pods were collected from the other centres.

The lower parts of the stem were dried and stored for several weeks, after which thirty pieces from each sample were surface sterilized and plated on potato-dextrose agar. A large number of the pieces proved to be sterile. A full list of the cultures isolated is given in Table III.

Table III

Fungi isolated from base of stems of plants (thirty pieces from each variety)

Variety	Locality	Pieces sterile	*Fusarium avenaceum*	*Fusarium culmorum*	*Fusarium solani Martii*	Other *Fusarium* spp.	*Botrytis cinerea*	Other fungi
Queen	Peterborough	13	13	0	0	1	0	3
Thomas Laxton	Rochester	19	0	0	2	3	0	6
Market Gardener	Rochford	10	5	0	0	2	1	12
Foremost	Evesham	17	0	0	1	10	0	2
Thomas Laxton	Rainham, Essex	14	0	0	0	8	1	7

(c) *Fungi isolated from diseased pods*

A few of the pods collected with the haulm from Evesham and Rochester showed a considerable amount of spotting typical of *Ascochyta*. They were dried and after a few weeks thirty seeds from the lightly infected pods were soaked, surface sterilized with silver nitrate and

Table IV

Fungi isolated from the seed in pods showing slight symptoms of disease, collected at Evesham and Rochester

Centre	No. of peas plated	Sterile peas	Peas yielding common moulds	Peas yielding *Ascochyta*	Peas yielding other fungi
Evesham	30	17	9	4	—
Rochester	30	2	24	—	4 (unidentified *Fusarium* spp.)

Table V

Fungi isolated from pods at Evesham and Rochester

Centre	Pod No.*	Fungus
Evesham (Plate I)	1	Unidentified fungus with sterile white aerial mycelium
	2	*Ascochyta pisi*
	3	*Ascochyta pisi*
	4	*Ascochyta pisi*
	5	*Ascochyta pisi* (?)
Rochester (Plate I)	6	Three species of *Fusarium*, unidentified
	7	Sterile fungus, resembling in colour and growth habit *Fusarium culmorum*
	8	Unidentified species of *Fusarium*
	9	*Botrytis cinerea*
	10	Unidentified fungus

* The pod numbers 1–10 refer to the position of the pods in Plate I.

plated on potato-dextrose agar. Five more heavily diseased pods from each centre, showing symptoms of *Aschochyta* and other fungi, were placed in a moist chamber for one week, when they showed abundant growth of fungi which were isolated. These ten pods were photographed before placing in the moisture chamber and are shown in Plate I. The fungi isolated from the seeds are listed in Table IV, and those from the pods in Table V.

It will be seen that there was a fairly heavy infection of *Ascochyta* at Evesham, while at Rochester this fungus was not secured, although the number of peas used was insufficient to say definitely that the fungus was not present. The results must not be interpreted as being an estimate of the relative abundance of *Ascochyta* at these centres, but serves to show that the method of surface sterilization used is quite suitable for obtaining this fungus from seed, and also to demonstrate that, under certain conditions, pea seed may harbour an appreciable fungal flora.

PATHOGENICITY OF ISOLATES

(a) *Isolates obtained from cotyledons of planted peas*

The cultures in this group are those referred to in Table II, obtained from cotyledons of seedlings of varieties Early Bird and Little Marvel. A representative of each fungus was tested in quadruplicate on 100 seedlings, variety Little Marvel, in unsterilized soil. Wooden boxes containing 25 seeds were used in a modification of Method II (*c*). The results obtained after 1 month's growth are given in Table VI.

Table VI

Results of inoculation of Little Marvel peas with isolates from diseased cotyledons of pea seedlings

Fungus	No. of seedlings from 100 seeds	Symptoms of rotting of plants on removal from soil after 1 month		
		Cotyledons	Roots	Stems
Fusarium culmorum from Early Bird	68	Severe	Moderate	Moderate
Fusarium sect. *Roseum* from Early Bird	5	Very severe	Severe	Severe
Fusarium sect. *Elegans* from Little Marvel	92	Slight	Trace	None
Penicillium species from Little Marvel	88	,,	,,	,,
Botrytis cinerea from Little Marvel	0	Very severe	—	—
Control	81	Slight	Trace	None

Fusarium culmorum*, *Fusarium of the section ***Roseum***, and ***Botrytis cinerea***, were obviously highly pathogenic and the last two named practically inhibited emergence of the seedlings. ***Fusarium*** section ***Elegans*** and ***Penicillium*** species were non-pathogenic.

(b) *Isolates obtained from foot-rot material*

Representatives of various fungi from the foot-rot material referred to in Table III were tested in duplicate for pathogenicity. The effects of these inoculations on the cotyledons, roots and stems are shown in Table VII.

Table VII

Effects of inoculating peas with foot-rot isolates

Source of isolate and fungus	Soil*	No. of seedlings from 20 seeds	Symptoms of rotting of plants on removal from soil after 21 days		
			Cotyledons	Roots	Stems
(1) Rochford:					
Fusarium avenaceum	+	0	Severe	—	—
	−	2	,,	Severe	Severe
Rhizopus species	+	20	None	None	None
	−	13	,,	,,	Trace
Fusarium species	+	18	,,	,,	None
	−	16	,,	,,	,,
Botrytis cinerea	+	0	Severe	—	—
	−	10	Moderate	Moderate	Moderate
Fusarium species	+	18	None	Slight	None
	−	14	,,	,,	Slight
(2) Rochester:					
Fusarium species	+	20	,,	,,	None
	−	8	,,	None	Slight
Dematiaceae	+	18	,,	,,	,,
	−	14	,,	Slight	,,
Fusarium solani var. *Martii* (?)	+	18	,,	,,	None
	−	10	,,	None	,,
(3) Rainham:					
Fusarium species	+	20	,,	,,	,,
	−	18	,,	,,	Slight
,,	+	20	,,	Slight	,,
	−	12	,,	,,	,,
Sterile mycelium	+	20	,,	None	None
	−	13	,,	,,	Slight
Fusarium species	+	19	,,	Slight	None
	−	11	,,	,,	,,
Dematiaceae	+	20	,,	None	,,
	−	16	,,	,,	,,
Fusarium species	+	20	,,	,,	,,
	−	5	,,	Slight	Slight
,,	+	20	,,	None	None
	−	17	,,	Slight	Slight
,,	+	20	,,	None	None
	−	20	,,	,,	,,
,,	+	20	,,	,,	,,
	−	12	,,	Slight	,,
,,	+	19	,,	None	,,
	−	17	,,	Slight	Slight
Macrosporium species	+	19	,,	None	None
	−	17	,,	Slight	Slight

* Sterilized (+), unsterilized (−).

Table VII (*cont.*)

Source of isolate and fungus	Soil	No. of seedlings from 20 seeds	Symptoms of rotting of plants on removal from soil after 21 days: Cotyledons	Roots	Stems
(4) Peterborough:					
Fusarium avenaceum	+	0	Severe	—	—
	–	0	,,	—	—
Fusarium species	+	18	None	None	None
	–	14	,,	Slight	,,
Fusarium avenaceum	+	0	Severe	—	—
	–	0	,,	—	—
,,	+	17	Moderate	Moderate	Moderate
	–	Not sown	—	—	—
(5) Evesham:					
Fusarium species	+	18	Moderate	Moderate	Moderate
	–	16	,,	,,	,,
Fusarium solani var.	+	19	None	Slight	None
Martii (?)	-	15	,,	None	,,
Fusarium species	+	20	,,	,,	,,
	–	15	,,	,,	,,
Control	+	20	,,	Slight	Slight
	–	17	,,	None	None
Control	+	20	,,	Slight	Slight
	–	14	,,	None	None
Control	+	20	,,	Slight	Slight
	–	18	,,	None	None
Control	+	20	,,	Slight	Slight
	–	16	,,	None	None

Of the twenty-seven isolates from rotted stems tested for pathogenicity, only six caused rotting of the cotyledons, stems and roots greater than that in the controls and these were all either *Fusarium avenaceum* or *Botrytis cinerea*. It will be noted that a fungus thought to be *Fusarium solani* var. *Martii* was not effective. A careful examination of this isolate showed that it could not be distinguished from *F. solani* var. *Martii* by morphological characteristics.

A culture of *Fusarium solani* var. *Martii* (?) was tested at a higher temperature (room temperature) in comparison with certain others of the above fungi in sterilized and unsterilized soil in duplicate tumblers of ten seeds, using 25% inoculum. Under these conditions a certain amount of rotting by this fungus occurred, together with marked blue coloration and rotting of the outer coating of the cotyledons, and considerable flecking of the stems. The reaction of this isolate was of a similar type but rather more severe than that produced by a culture of *F. solani* var. *Martii* supplied by courtesy of the Long Ashton Research Station through Mr Hickman. The fungus did not lower the percentage germination and could be said at most to be a weak parasite on pea seedlings.

(c) *Miscellaneous stock cultures*

Cultures of widely differing groups of fungi from a stock collection were tested in unsterilized soil, five replicates being used. Results obtained with the fungi in this group tested in unsterilized soil are shown in Table VIII.

Table VIII

Effects of inoculating peas with miscellaneous fungi

Fungus	No. of seedlings from 50 seeds	Symptoms of rotting of plants on removal from soil: Cotyledons	Roots	Stems
Rhizoctonia solani from rhizomes of *Agropyron repens*	46	Slight	Slight	Slight
Fusarium avenaceum from *Triticum*	48	Moderate	,,	Moderate
Fusarium culmorum from *Triticum*	34	,,	,,	Slight
Fusarium culmorum from *Dianthus*	33	,,	,,	,,
Fusarium species from *Vicia faba*	42	,,	Moderate	Moderate
Fusarium bulbigenum	46	None	Slight	Slight
Fusarium anguioides	48	,,	,,	,,
Fusarium species from *Tulipa*	48	,,	,,	,,
Botrytis cinerea from *Lactuca*	0	Severe	—	—
Ascochyta pisi from *Pisum*	46	Moderate	Slight	Slight
Botrytis cinerea from *Pisum*	0	Severe	—	—
Fusarium avenaceum from *Pisum*	30	,,	Severe	Severe
Cladosporium herbarum	48	Slight	Slight	Slight
Helminthosporium avenae from *Avena*	44	,,	None	None
Fusarium nivale from *Poa*	46	,,	,,	Slight
Penicillium digitatum	45	,,	Slight	,,
Fusarium culmorum from *Triticum*	35	Severe	Moderate	Moderate
Fusarium culmorum from *Callistephus*	39	,,	,,	,,
Fusarium Graminearum from *Triticum*	6	,,	Severe	Severe
Fusarium conglutinans from *Callistephus*	48	Slight	None	Slight
Fusarium Dianthi from *Dianthus*	46	,,	Slight	None
Fusarium species from *Tulipa*	47	Severe	Moderate	Moderate
Botrytis tulipae from *Tulipa*	47	Slight	Slight	Slight
Botrytis cinerea from *Rosa*	10	Severe	Severe	Severe
Helminthosporium sativum from *Triticum*	34	,,	,,	,,
Botrytis cinerea from *Lactuca*	0	,,	—	—
Helminthosporium teres from *Hordeum*	50	Slight	Slight	Slight
Botrytis cinerea	22	Severe	Severe	Severe
Fusarium coeruleum	49	Slight	Slight	Slight
Penicillium italicum	44	,,	None	None
Sclerotinia species (?) from *Lactuca*	0	Severe	—	—
Ophiobolus heterostrophus from *Oryza*	44	Slight	Severe	Severe
Control	47	Slight	Slight	Slight
Control	45	,,	None	,,

This group of fungi has again yielded interesting results. The fungi showing pathogenicity were:

Fusarium avenaceum from *Triticum.*
Fusarium culmorum from *Triticum.*
Fusarium culmorum from *Dianthus.*
Fusarium species from *Vicia faba.*
Botrytis cinerea from *Lactuca.*
Ascochyta pisi from *Pisum.*
Botrytis cinerea from *Pisum.*
Fusarium avenaceum from *Pisum.*
Fusarium culmorum from *Callistephus.*
Fusarium Graminearum from *Triticum.*
Fusarium species from *Tulipa.*
Botrytis cinerea from *Rosa.*
Helminthosporium sativum from *Triticum.*
Sclerotinia species(?) from *Lactuca.*
Ophiobolus heterostrophus from *Oryza.*

DISCUSSION

According to the literature a number of species of *Fusarium*, and in addition to some extent other fungi (*Mycosphaerella pinodes*, *Ascochyta pinodella*, *Aphanomyces euteiches* and *Rhizoctonia solani*), cause root-rot or foot-rot of peas in various parts of the world. It is also evident from the present work that many fungi are associated with the rotting of stems of mature plants, many of which cannot initiate rotting in young seedlings.

The results of these experiments show that in addition to those fungi recorded as occurring on pea seed in the literature, a number of fungi obtained from pea stems and from other sources cause severe rotting of the cotyledons. Of those tested, the following proved pathogenic:

Botrytis cinerea from *Lactuca*, *Rosa*, and elsewhere.
Fusarium avenaceum from *Triticum.*
Fusarium culmorum from *Triticum*, *Dianthus* and *Callistephus.*
Fusarium Graminearum from *Triticum.*
Fusarium species (unidentified) from *Vicia faba* and *Tulipa.*
Helminthosporium sativum from *Triticum.*
Sclerotinia species(?) from *Lactuca.*

Ophiobolus heterostrophus causes severe spotting without rotting of cotyledons.

It is clear from the present research that rotting of pea seeds in soil may be due to various fungi, and in nature presumably it will frequently be due to the combined effects of several. The greater pathogenicity of several fungi such as *Fusarium avenaceum*, *F. culmorum* and *Botrytis cinerea* in conjunction with their frequency of isolation and well-known wide distribution in soil suggest their importance. Stress need not be laid on the fact that isolates believed to be *Fusarium solani* var. *Martii* appeared only slightly pathogenic, as Snyder & Walker (1935) have stated that the only satisfactory means of distinguishing between forms of *F. solani* is by tests for parasitism on different hosts. That it can be an important parasite of peas has been fully established elsewhere.

The severe losses encountered in field experiments where seed dressings were not used can be put down to rotting of cotyledons, roots and stems caused by *Fusarium avenaceum* and other fungi probably including *F. culmorum* and *Botrytis cinerea*, but not seed-borne *Ascochyta pisi* and *Mycosphaerella pinodes*. The disease characteristic of that caused by the first three fungi has not yet been appreciated to the full, and it is possible that poor stands of peas (resulting in poor crops) are more frequently due to these fungi than to *Mycosphaerella* and *Ascochyta*, which can readily be detected on the seed and are, in fact, eliminated to a great extent by discarding poor seed.

The rotting of cotyledons of pea seed in the soil may be looked upon as a complex disease in which more than one fungus may be playing a part, but as far as the evidence goes, these fungi are distinctly parasitic in nature and the cotyledons can probably withstand an abundance of common moulds.

Summary

1. Poor stands of peas due to fungal rotting were observed in field experimental plots in widely separated pea-growing districts.

2. The rotting was not due to seed-borne *Ascochyta pisi* or *Mycosphaerella pinodes* which were apparently absent from the seed.

3. Isolations from surface sterilized cotyledons of several varieties of peas grown in sterilized and unsterilized soil showed an abundance of common moulds and several pathogenic fungi, namely: a *Fusarium* of the section *Roseum*, *Fusarium culmorum* and *Botrytis cinerea*.

4. Isolations from surface sterilized rotting stems from five experimental centres at harvest time indicated the presence of a great variety of fungi including a number of species of *Fusarium*. Many of these, including all the common moulds such as *Penicillium*, were non-pathogenic

to the cotyledons. *Fusarium avenaceum* was found to be highly pathogenic to the cotyledons; *F. solani* var. *Martii* showed some signs of causing rotting; *Botrytis cinerea* was highly pathogenic. Several non-pathogenic species of *Fusarium* were obtained.

5. Tests with numerous stock cultures show high pathogenicity of the following fungi to pea cotyledons:

Botrytis cinerea from *Lactuca*, *Rosa* and elsewhere.
Fusarium avenaceum from *Triticum*.
Fusarium culmorum from *Triticum*, *Dianthus* and *Callistephus*.
Fusarium species from *Vicia faba* and *Tulipa*.
Helminthosporium sativum from *Triticum*.
Ophiobolus heterostrophus from *Oryza*.
Sclerotinia species from *Lactuca*.
Fusarium Graminearum from *Triticum*.

6. It is suggested that loss of stand in pea crops due to rotting of the cotyledons by these and other fungi is probably more important than diseases caused by *Ascochyta* and *Mycosphaerella* and foot-rots in the late stage.

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EXPLANATION OF PLATES I AND II

PLATE I

Pods of peas collected at harvest time. Pods showing fungi as follows:

Figs. 1–5. Evesham collection

Fig. 1. Unidentified fungus with sterile white aerial mycelium.
Fig. 2. *Ascochyta pisi* and sterile fungus.
Fig. 3. *Ascochyta pisi*.
Fig. 4. *Ascochyta pisi*.
Fig. 5. *Ascochyta pisi*(?).

Figs. 6–10. Rochester collection

Fig. 6. Three unidentified species of *Fusarium*.
Fig. 7. Sterile fungus, resembling in colour and growth habit *Fusarium culmorum*.
Fig. 8. Unidentified species of *Fusarium*.
Fig. 9. *Botrytis cinerea*.
Fig. 10. Unidentified fungus.

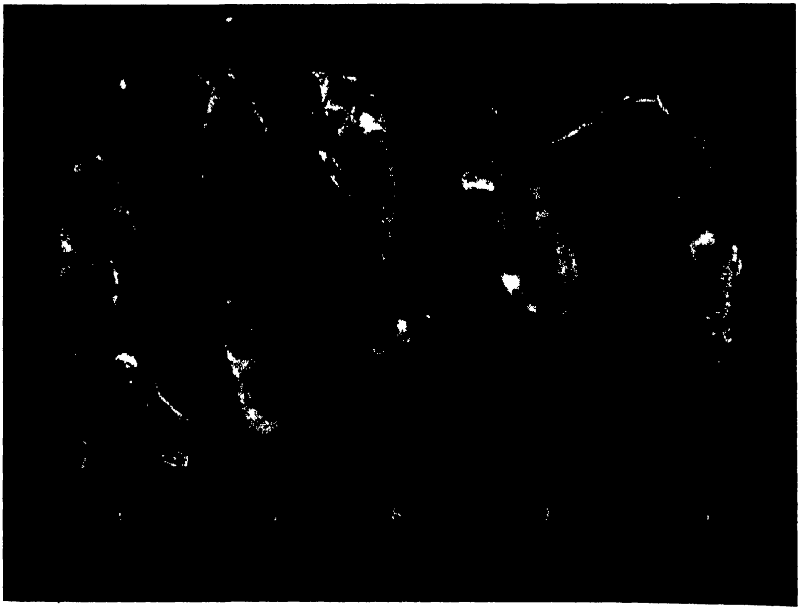
PLATE II

Peas planted in soil heavily contaminated with *Fusarium avenaceum*

Fig. 1. Seed treated with a mercurial dressing.
Fig. 2. Untreated seed.

(*Received* 15 *July* 1937)


5



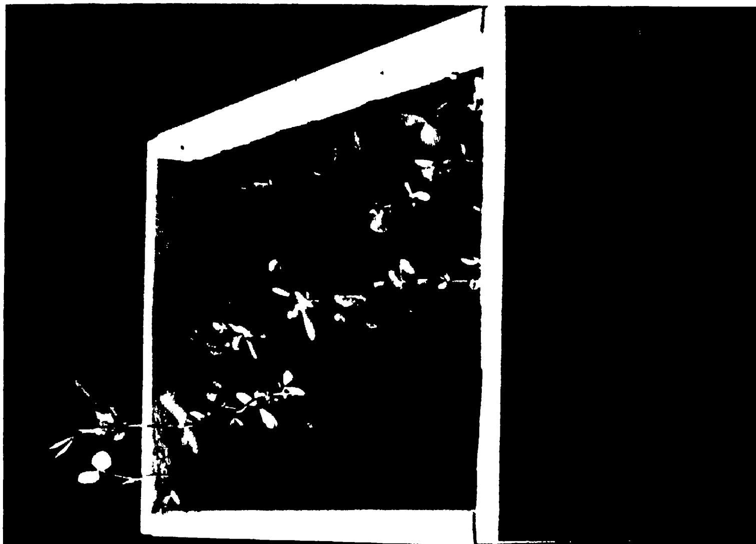


Fig. 2.

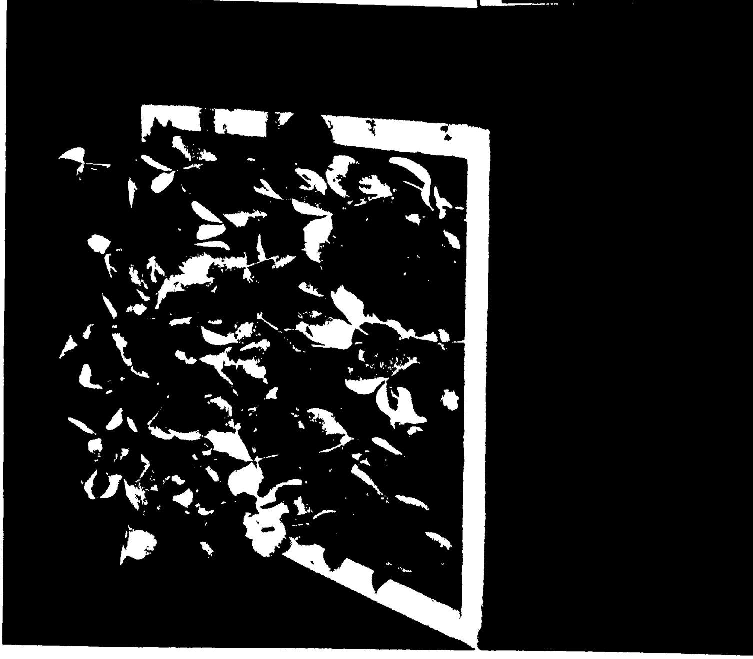


Fig. 1.

PADWICK.—Complex Fungal Rotting of Pea Seeds (pp. 100–114)

A DISEASE OF THE VIOLA CAUSED BY *RAMULARIA DEFLECTENS*

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(With Plate III and 2 Text-figures)

VIOLA plants of the variety Kate Blyth badly infected with *Ramularia* were received from Coventry in February 1934. The plants were set out in boxes and the fungus grown in culture.

DESCRIPTION ON HOST

On the infected plants the fungus was confined to the leaves. Dark-coloured lesions, which as they grow older develop a white mycelium in the centre, start at the edge of the lamina and gradually spread inwards, often in the form of a semicircle. Often these lesions run together until the whole leaf, both on the upper and lower side, is covered with a dry white weft of hyphae. The conidiophores are branched, septate and toothed at the apex. The conidia vary from 6·9 to 24·1 μ in length and from 2·2 to 3·4 μ in breadth.

The fungus agrees with *R. deflectens* Bresadola (Lindau, 1907), the spore size being given as 18–40 × 5–7 μ. Although this is larger than the above measurements the description agrees otherwise, hence it has not been thought advisable to make a new species. In transverse sections of the leaves the black sclerotia-like bodies lie immediately under the epidermis. They measure from 40 to 70 μ in diameter and are found to vary in shape and size. The smaller ones are spherical, while the larger assume a flask shape with a definite papilla which protrudes through the ruptured epidermis. These flask-shaped bodies have hyphae protruding from the papilla and small spores, 3·4–5·4 × 2–2·2 μ, were found attached to their ends as Laibach (1921–2) observed in *R. knautiae* (Pl. III, fig. 1). These bodies are composed of three to four outer layers of cells with thick walls, while the interior is made up of smaller and thinner walled cells which appear lighter in colour. In a few cases small spores have been detected in the centre. These structures are evidently a form of pycnidia which do not, however, produce pycnospores in any great

number. Laibach in dealing with *R. knautiae* considers that these sclerotia are a form of perithecia which, for some reason, have been hindered in their formation and whose development has been led into another channel at an early stage. The fact that small spores have been found both within them and emerging from the papillae seems to suggest that they are really potential pycnidia. Perithecia have not been found on any of the plants examined.

Cultural methods

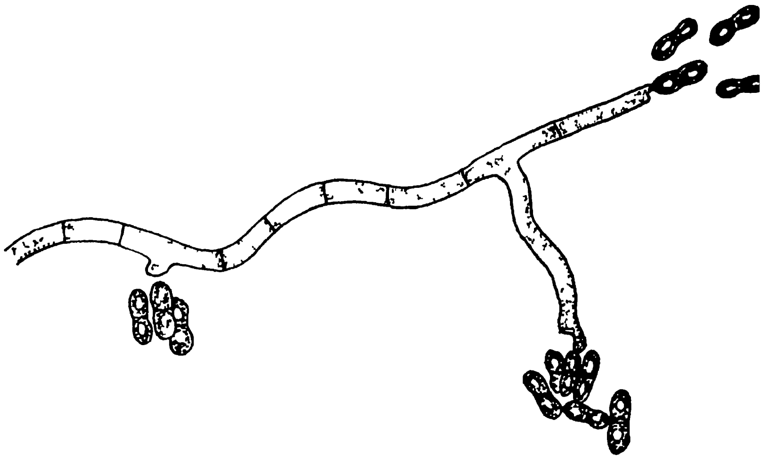
Cultures on plain agar were made from the conidia on the leaf and, after several days, monospore cultures were made from these on malt agar. The following media were found to be the most successful: malt agar, oat, pea, and bean agar.

Cultural characters

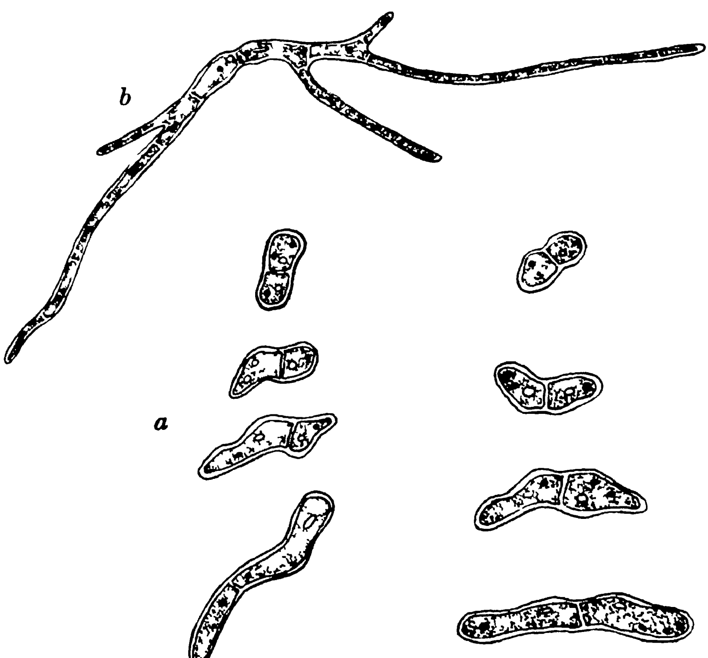
The optimum growth of the mycelium occurred on malt agar at 17° C. It was found by experiment that light does not affect the growth of this fungus.

(*a*) *Conidia.* In culture the conidia are cylindrical, laterally compressed and slightly bent. The mature spores are two-celled, while the youngest have only one cell. Lactic acid cotton blue is quickly absorbed by these conidia, but in the centre of each cell a clear vacuole is seen. When stained with Sudan III these vacuoles assume a bright red colour showing that they are oil drops. The conidia measure $5\text{–}11{\cdot}4 \times 2{\cdot}5\text{–}3{\cdot}5\,\mu$ with an average of $8{\cdot}4\,\mu$ (sixty measurements). Their attachment to the dentate septate conidiophore is terminal and as each new conidium is formed it causes the older conidia to be displaced laterally (Text-fig. 1). These conidia germinate within 24 hr. and either one cell or both cells may produce a germ tube (Text-fig. 2*a*). As growth proceeds cross septa are formed and branches from the germ tube are given off (Text-fig. 2*b*).

(*b*) *Pycnidia.* In multispore cultures after 3–4 months and in very old revived monospore cultures pycnidia were formed. The hyphae at different points in the culture took on a greenish tinge and when examined were seen to be greatly enlarged and clubbed together, while fusions between them had taken place. Finally this irregular green mass became black and formed the primordium of a pycnidium. The pycnidia possess a great range both in size and form, they vary from spherical to flask-shape and may measure from 100 to $200\,\mu$ in diameter. In section they show three outer layers of thick dark-walled cells, while the centre may be composed of light-coloured cells as in the young stage or may, when mature, be filled with spores which measure $4{\cdot}1\text{–}6{\cdot}6 \times 2\text{–}2{\cdot}5\,\mu$. The



Text fig 1 Conidiophore bearing conidia Camera lucida × 1700



Text fig 2 *a*, germinating conidia Camera lucida × 2333 *b*, germinating conidium, later stage Camera lucida × 1750

pycnospores are borne terminally, on extremely fine filaments which arise from a row of cells next to the innermost layer of thick-walled cells. The shape of the mature pycnidium was followed through in a series of microtome sections and was found to be flask-shaped with a definite ostiole (Pl. III, fig. 2). In culture the pycnidia were formed in zones as Klebahn (1918) noted in *R. hieracii*. A few pycnidia are usually formed at the point of inoculation, and these are surrounded by a pale zone in which no pycnidial formation takes place. Outside this a dark zone occurs in which pycnidia are present. As this zonation, which occurs throughout the culture, is present in different media and under different conditions of light and temperature, it would seem to be due to the constitution of the fungus and not to the environment (Pl. III, fig. 3). When microtome sections of the agar slope were examined it was noted that on the surface of the medium the pycnidia formed clusters of three to four, while within the medium they were borne singly. The following experiment proved that these pycnidia belong to the *Ramularia* and that their presence is not due to contamination. Pycnidia were washed in a solution of 1/1000 mercuric chloride for 3 min., removed to sterile water and washed thoroughly. Single pycnidia were then removed by means of a dry sterile needle, inoculated into tubes of malt agar and incubated at 17° C. After 5–6 days hyphae were observed growing from the circumference of the pycnidia. In two out of the six thus treated, pycnidia were reformed in the culture, while in the remaining four cultures pycnidia were absent, while conidia were found to be plentiful. The above experiment was repeated, but this time a 1 % HCl solution for 5 min. was substituted, conidia when thus treated lost their power of germination, and so it is assumed that adhering conidia were killed as in the last experiment. Within 17 hr. a fine growth of hyphae was observed growing out from the pycnidia, while after 4 days all the cultures thus produced had formed pycnidia, and a marked zoning in the culture. The rapid germination of the pycnidia may be due to the stimulation caused by the action of the HCl upon the cells. In one or two cases pycnidia were placed upon a dry slide in a drop of sterile water and then burst by a sterile needle. The escaping pycnospores were then removed and put on to tubes of malt agar. After 3–4 days growth was observed. One week later the culture was found to contain typical conidia while pycnidia were subsequently formed.

In appearance they resemble very closely the so-called sclerotia of the leaf, and the following points of similarity were noted:

(1) The wall layers of both are identical in form.

(2) When seen in section the ripe sclerotia and pycnidia have a flask-shape with a definite opening.

(3) The pycnospores measure 4·1–6·6 × 2–2·5 μ, while the spores borne on the hyphae of the papillae measure 3·4–5·4 × 3–2·2 μ. Thus it is seen that there is a close approximation between the pycnidia formed in culture and the sclerotia in the tissue of the host.

(*c*) *Monospore cultures.* In monospore cultures after 3–4 days a white frill of hyphae is seen round the point of inoculation. This gradually increases in size until by the end of 7 days a small, circular clump of hyphae, which is floccose in appearance and projects from the substratum, is formed. The hyphae grow over the surface of the agar forming conidia freely, giving the culture a cream colour and forming a thickened rim at the growing edge. After 28–30 days the surface of the agar is entirely covered by the growth of the fungus, and conidial pustules are beginning to form from the substratum. These pustules are pale cream in colour and, in culture of 2 months, they are dotted over the entire surface of the slope. Monospore cultures on oat agar and on bean agar show the same appearance but the conidia are formed in greater abundance, the whole surface becoming covered by a deep cream coloured layer of spores. Similar results were obtained by growing the fungus on small sterilized pieces of Viola stem.

In cultural experiments the following facts have come to light:

(1) A monospore culture produced conidia but no pycnidia. After a resting period of 9 months this dried culture was revived by pouring fresh malt agar down the side of the tube. Pycnidia were now produced in abundance within 5 days.

(2) A multispore culture from the same culture as the above monospore produced pycnidia as well as conidia within 3 months.

(3) A subinoculation from this multispore culture continued to produce pycnidia, while a monospore culture from it formed conidia but no pycnidia.

It is obvious that pycnidia are formed both from monospore and from multispore cultures. Their formation is dependent on the lapse of some considerable period of time, but it seems that when pycnidial formation has once commenced it will continue even when subcultures are made. The period required for pycnidial formation appears to depend on the amount of the material used in making the culture. In the case of the multispore culture, where much material is transferred, the period is short, while on the other hand, in the case of the monospore culture

the period is considerably lengthened. Possibly this may be explained as the accumulation of some substance during the growth of the fungus. The whole question of the formation of the pycnidia seems to centre round the problem of nutrition, which is closely linked up with a time factor.

Perithecia. Perithecia were not obtained in culture. Various methods such as growing on special media, freezing and the addition of fungal extracts were tried but they all failed to induce perithecial formation. The perfect stage of the genus *Ramularia* does not appear to be found in culture. Laibach (1921–2), working on *R. knautiae*, and Klebahn (1918) on *R. hieracii*, both failed to find the perfect stage in culture, although it occurred in both these cases on the host plant and proved to be a species of *Mycosphaerella*.

Infection experiments

Infection experiments were carried out in August 1934. Viola plants of the varieties Aberdeen Blue and Mosley's Perfection were inoculated with a spore suspension from a monospore culture of *R. deflectens*. The plants were kept in a cold frame, but in no case were positive results obtained. By the beginning of October 1934 it was found that the original diseased plants of the variety Kate Blyth had become healthy and had lost all signs of the disease. Cuttings were made from these plants. In February the leaves of these plants were sterilized and then four were kept as controls while the other four were inoculated as above. After a week or two all four inoculated plants showed the presence of the fungus on the leaves (Pl. III, figs. 4, 5), while the four controls remained healthy. The spores measured $9{\cdot}5\text{–}35 \times 4{\cdot}7\text{–}5\,\mu$ $(22 \times 5\,\mu)$. Monospore cultures were made from these conidia and in culture the spores measured $8{\cdot}9\text{–}23 \times 4{\cdot}5\text{–}5\,\mu$.

It appears that there is a tendency for the conidia to be larger on the host than in culture. The failure of the infection experiments in August may have been due to the variety used, or to the fact that the plants are more resistant to disease at that time of the year.

Conclusion

Ramularia deflectens appears to be a weak parasite which only attacks the Viola in early spring. A cool moist atmosphere appears to favour the growth of the fungus. As perithecia have been found neither on the fresh material nor in culture, the correct systemic position of this

Fig 1

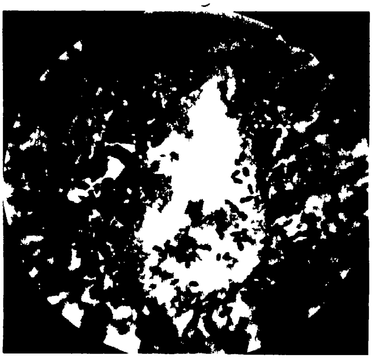


Fig 2

Fig 4

Fig 3

Fig 5

Ramularia cannot be decided on. The presence of pycnidia seems, however, to warrant its withdrawal from the Moniliales.

SUMMARY

1. Viola plants, of the variety Kate Blyth, which were badly infected with the fungus *Ramularia deflectens*, were received from Coventry in the early spring.
2. Sclerotium-like bodies were found on the leaves.
3. Pycnidia containing pycnospores were obtained in culture.
4. By comparison it is seen that these sclerotium-like bodies are really pycnidia which have not developed fully.
5. The formation of pycnidia in culture depends on nutrition.
6. Perithecia are found neither on the plant nor in culture.
7. In the early spring positive results from infection experiments are obtained.

The writer wishes to thank Dr Malcolm Wilson for his invaluable assistance and helpful criticism throughout the work.

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EXPLANATION OF PLATE III

Fig. 1. Transverse section. Leaf of viola showing pycnidium. Camera lucida. × 4000.
Fig. 2. Transverse section. Culture. Pycnidium showing ostiole. Photomicrograph. × 2000.
Fig. 3. Zoning in culture due to pycnidial formation. × 1½.
Fig. 4. Infected plant showing presence of *R. deflectens*. × ½.
Fig. 5. Leaf of viola showing lesion caused by *R. deflectens*. × 6.

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STUDIES ON APHIDES INFESTING THE POTATO CROP

VI. APHIS INFESTATION OF ISOLATED PLANTS

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The previous papers in this series have presented the results of studies on the aphis population of the potato crops in districts contrasting in the degree of spread of virus diseases (Davies, 1932, 1934, 1937; Davies & Whitehead, 1935) and also on the factors affecting migration of winged aphides (Davies, 1935–7; Davies & Whitehead, 1935). The present investigations were planned to ascertain the extent of the migrations of winged aphides, their ability to detect individual potato plants, the development of aphides on such units and the extent to which virus diseases were carried to these isolated plants.

Method

Centres were selected in districts contrasting in the usual rate of increase in virus infection among introduced healthy stocks of potatoes. Four centres were in the eastern part of North Wales where the spread of disease has been rapid, viz. Holywell, Dyserth and Hawarden in Flintshire, and Abergele in Denbighshire. Two were selected in South Caernarvonshire where there has been no appreciable increase in virus infection during the last nine years, viz. Madryn and Aberdaron. One other centre was at the College Farm near Bangor, which is intermediate in character but where the sources of possible infection were great since the experimental potatoes were planted in the same field as the infected plots of the virus museum.

At each centre, one hundred (or fifty) half-tubers of healthy stocks of Arran Comrade potatoes were planted separately in a root crop, with at least 6 yd. between the potato plants along the drill and 10 yd. across the drills of the mangold or sugar-beet crop. The corresponding half-tuber controls were planted at the College Farm in order to ascertain the health of the experimental plants; no virus symptoms appeared in these controls. Had it been practicable the experimental half-tubers would have been

grown on fallow land at the different centres in order to maintain complete isolation but, this being impossible, mangold and sugar beet were selected because at the time of appearance of the potato plants above ground, as well as at the time of migration of aphides, the potato units would be well separated and conspicuous as isolated plants. It was realized that *Myzus persicae*, the chief insect vector of virus diseases, is found occasionally on mangolds, but it is rarely present in considerable numbers before the foliage is large. Further, since the prevention of migration from one potato unit to another by crawling Apterae was being aimed at, the presence of another plant on which the migrating aphides might remain was no disadvantage. Cereal crops were not selected because they would have quickly smothered the potato foliage.

The half-tubers were planted at all centres in Flintshire on 10 May 1935, and in South Caernarvonshire 4 days later. The plants were first examined on 14 June when the majority were just through the soil and none was more than 4 in. high. The infestation of each species of aphis found was recorded from three leaves of each plant. When no aphides were found on the three leaves, which were always a random selection, the record was given as nil, but the whole plant was then examined for the presence of *M. persicae* and, if found, the number was recorded in brackets. These data were obtained at intervals during the season. In the autumn the progeny of each plant was separately lifted, boxed and removed to the College Farm, where it was stored until planted in the spring of 1936.

THE APHIS INFESTATION OF ISOLATED PLANTS AT DIFFERENT CENTRES

In an investigation of this nature it is important to study the history of each plant separately. The complete records from each centre are therefore presented, including the aphis population found at successive dates on each plant and the state of health of the progeny in the following year.

Centre I (Berthymaen, Holywell, Flintshire)

The aphis population on the potato crop at this centre has been observed since 1928 by the standard method of counting the population per 100 leaves, selected at random as the crop was traversed to and fro. The following figures give the "index-figure" of infestation for each year: 1928 (360, all spp.), 1929 (1910, all spp.), 1930 (550, *M. persicae*), 1931 (118, *M. persicae*), 1932 (1103, *M. persicae*), 1933 (848, *M. persicae*), 1934 (520, *M. persicae*), and 1935 (210, *M. persicae*). The rate of increase of

virus diseases at this centre was rapid, amounting to 33 % in 2 years (Whitehead *et al.* 1932.)

The isolated half-tubers at this centre were planted along the mangold rows, the nearest tubers being 40 yd. from the general farm crop of potatoes which occupied the same field. The hundred healthy, experimental half-tubers were planted on 10 May and seventy-five were above ground on 14 June but were not more than 4 in. high. The infestation record and subsequent health of the progeny of these plants are given in Table I.

Aphis infestation. The ability of winged aphides to detect and colonize isolated plants proved to be remarkable for, as can be seen in Table I, on 14 June, when the plants were very small, 61 % of those above ground were already infested with *M. persicae.* A fortnight later, on 28 June, when practically all plants had appeared above ground though none was more than 6 in. high, 98·9 % were infested with this species and at least 29 % carried *Macrosiphum gei* Koch. Further, there were nineteen *winged M. persicae* actually present on the forty-six infested plants (only three leaves on each plant being examined) on 14 June. It seems reasonable to assume therefore that the wingless forms found on the other plants had been produced by winged migrants which were either on leaves not examined or had again moved on to other plants. In fact, in most cases the nymphs, when present alone, were so small that it would have been impossible for them to have crawled any great distance across arable land. It follows therefore that, since the half-tubers were free from aphides when planted and no aphides were found on the seedling mangolds, the migrating winged aphides could detect and colonize isolated plants with ease, even in a season when the general aphis population was low in this district.

Spread of virus diseases by winged aphides. The health of the 100 half-tubers planted at this centre was confirmed by growing the corresponding halves at the College Farm, there being no symptoms of virus infection on these latter plants. The very considerable migration of winged aphides to the isolated plants at Berthymaen, shown in Table I, emphasized the importance of ascertaining the extent to which these migrants had infected the plants with virus diseases. The progeny of the plants was lifted in October 1935, removed and stored separately at the College Farm until spring, when four tubers of each unit were planted to ascertain their health. An examination of the last column in Table I indicates the remarkable amount of virus disease (94·6 %), particularly leaf-roll, which had been carried to the isolated plants in 1935; it may be added that in

Table I

Aphis population on isolated plants and evidence of virus transmission

Centre I (Berthymaen, Holywell, Flintshire). Potatoes isolated in mangold crop with farm potato crop 40 yd. distant in same field

	Aphides present on three leaves on			
Plant	14. vi. 35	28. vi. 35	26. vii. 35	Health of progeny 1936
1	3p	1g (p)	2p	Leaf-roll
2	1p*w*	1g, 10p	(p)	,,
3	–	1p	(p)	,,
4	1g, (p)	1p	(p)	Healthy
5	–	1p*w*	0	Leaf-roll
6	2p*w*	5p	1p	,,
7	–	9p	0	,,
8	1p*w*	12p	10p, 14g	,,
9	–	–	–	–
10	1p, 1p*w*	1p*w*, 3p, 1g	(p)	Leaf-roll
11	3p	(p)	(p, g)	,,
12	0	1p*w*, 3p	(p)	,,
13	(p)	(p)	(p)	,,
14	–	1p*w*, 5p	0	,,
15	1p*w*	1p	0	,,
16	1p*w*	1p	(p)	,,
17	1p*w*	4p, 1g	1p	,,
18	1p*w*	1p*w*, 2p	4p	,,
19	–	(p)	(p)	Healthy
20	–	1p	0	Leaf-roll
21	–	1p	0	,,
22	1p*w*	1p*w*, 6p, 1g	14p, 3g	,, + Interveinal mosaic
23	(p)	1p	(p)	,,
24	2p*w*	2p	(p)	,,
25	–	1p*w*, 2p	1g	Missing
26	0	1p, 1g	(p)	Leaf-roll
27	1p	1p, 2p*w*, 1g*w*	1p, 3g	,, + mosaic
28	(p)	2p*w*, 6p	(p)	,,
29	(p)	4p	(g)	,,
30	1p*w*	(p*w*)	(p)	Missing
31	0	1p, 1g	3p	Leaf-roll
32	1p	1p*w*, 1p	(p)	,,
33	1p	1p*w*, 5p	(p)	,,
34	0	8p, 2g	(p)	,,
35	(p)	(p)	0	,,
36	1p	2p	1p	Mosaic
37	1p	2p*w*, 2p	1g	Leaf-roll
38	(p)	10p	1p, 1g	,,
39	0	1p*w*, 8p	2g	,,
40	(p)	5p	(p)	,,
41	(p)	9p	1g	,,
42	1p*w*, 1p, 1g	2p*w*, 7p	(p)	,,
43	1p	13p	1g	Missing
44	0	1p*w*, 4p, 1g*w*	0	Leaf-roll
45	1p	13p	(p)	,,
46	–	6p	(p)	,,
47	–	(p)	0	Missing
48	1p	5p	1g	Leaf-roll
49	–	1p*w*, 5p	(p)	,,
50	0	2p	(g)	,,

Table I (*cont.*)

Plant	Aphides present on three leaves on 14. vi. 35	28. vi. 35	26. vii. 35	Health of progeny 1936
51	0	13p	(g)	Leaf-roll
52	1p*w*	1p	0	,,
53	(p)	1p	(p)	,,
54	–	1p*w*, 3p	(g)	,,
55	–	1p, 4g	3p	,,
56	1p	1p	3p	,, + interveinal mosaic
57	–	6p	2g	,,
58	–	20p	2p	,,
59	–	5p	(p)	,,
60	0	1p	2p	,,
61	0	15p	(p)	,,
62	0	3p	0	,,
63	1p	16p	6p, 2g	,,
64	1p	24p	(p)	Missing
65	0	7p	(p)	Leaf-roll
66	–	(g)	(p)	,, (one tuber)
67	0	3p	3p	Healthy
68	–	–	1p	Leaf-roll
69	1p	6p, 4g	(p)	,,
70	1p	12p, 6g	1g	,,
71	0	5p, 1g	1p	Missing
72	0	6p, 2g	(g)	Leaf-roll
73	0	4p, 2g	(p)	,,
74	0	1p*w*, 1p	11p	,,
75	1g, (p)	3p	(p)	Healthy
76	0	4p	(p)	Leaf-roll
77	0	2p, 1g*w*, 1g	(g)	,,
78	0	6p	1p	,,
79	0	12p, 3g	1g	,,
80	0	(p)	1p, 2g	,,
81	1p	2p, 5g	0	,,
82	0	3p	0	,,
83	–	(p)	0	,, (one tuber)
84	1p	1p*w*	1g	Healthy
85	–	6p, 3g	(p)	Leaf-roll
86	0	1p, 4g	6p	,,
87	1p*w*	1g, 5p	(p)	,,
88	1p*w*	7p	1g	,,
89	–	3p, 3g	(g)	,,
90	1p	11p	0	,,
91	1p*w*	5p, 3g	3g	,,
92	–	5p	(p)	,,
93	0	5p, 6g	1p	,,
94	1p	15p, 1g	(p)	,,
95	–	13p, 2g	1p	,,
96	1p	1p	(p)	,,
97	1p*w*, 1p	(p)	(p)	,, (one tuber with interveinal mosaic)
98	0	9p, 4g	(p)	,,
99	0	2p	11p	,,
100	–	3p	0	,,

p = *Myzus persicae* (wingless). p*w* = *Myzus persicae* (winged). g = *Macrosiphum gei* (wingless). g*w* = *Macrosiphum gei* (winged). Brackets, e.g. (p) = *M. persicae* present on plant but not on the three leaves taken for count. – = Plant not above ground.

almost every case all four tubers from each unit plant showed symptoms of infection. Eighty-three out of the ninety-three plants tested (seven isolated plants being missing at lifting time) had the progeny infected with leaf-roll, three other plants had leaf-roll and interveinal mosaic, one had leaf-roll together with simple mosaic and there was one case of simple mosaic alone, leaving only five plants with healthy progeny.

This result was surprising in view of the fact that previously (Davies & Whitehead, 1935), when winged aphides had been collected on arrival on healthy stocks at this centre and were transferred to the laboratory, it was found that only a negligible number were infected with virus diseases; this was confirmed in 1935 (cf. p. 138 below). It would appear, therefore, that whereas the initial migrants arriving on potatoes are largely free from virus infection, there is a considerable spread of disease by winged forms at a later date. The uniform infection of all, or nearly all, tubers on each plant also points to such spread occurring fairly early in the growing season, though no doubt it is continued by subsequent winged generations produced on the potato crop. At this centre the nearest source of virus infection was a crop of Arran Comrade potatoes, *ex* Scotland, some 40 yd. distant from the isolated plants and containing 5 % leaf-roll. On a neighbouring farm 3·8–6·4 % of leaf-roll occurred in different varieties.

Centre II (Ty Mawr, Abergele, Denbighshire)

This centre, in common with the last one, had been discarded for seed-potato production, under the North Wales Scheme, owing to the rapid spread of virus infection in the stocks. The index figure of the aphis population since 1928 was as follows: 1928 (146, all spp.), 1929 (354, all spp.), 1930 (701, *M. persicae*), 1931 (130, *M. persicae*), 1932 (525, *M. persicae*), 1933 (520, *M. persicae*), 1934 (no record), and 1935 (123, *M. persicae*).

At this centre, also, the general crop of potatoes (about a quarter of an acre) was about 40 yd. away from the nearest isolated plant and in the same field. This main crop contained 4 % leaf-roll and 1 % mosaic. Sixty half-tubers were planted in a mangold crop and at the same intervals as at centre I; the control halves being grown, as before, at the College Farm. Two of the experimental plants were missing at lifting time, and the complete record of the remaining fifty-eight plants is given in Table II.

Counts of the aphis population were taken on only two occasions, since it was found on the second date that practically all plants were

Table II

Aphis population on isolated plants and evidence of virus transmission

Centre II (Ty Mawr, Abergele, Denbighshire). Potatoes isolated in mangold crop, with ¼ acre farm potato crop in same field, distant 40 yd.

Plant	Aphides present on three leaves on 27. vi. 35	Aphides present on three leaves on 25. vii. 35	Health of progeny 1936
1	1p	(p)	Mosaic
2	1p	(p)	,,
3	–	(p)	Leaf-roll + mosaic
4	1p	2p	Mild mosaic
5	1p	(p)	,,
6	1p, 2g	(p)	Leaf-roll
7	–	(p)	Crinkle
8	1g	5p	,, (two tubers)
9	0	(p)	Mild mosaic
10	0	(p)	,,
11	1p*w*	(p)	Crinkle
12	1p*w*	7p	Leaf-roll
13	0	(p)	Healthy
14	4p, 2g	(g)	,,
15	5p, 1g	(p)	,,
16	1p	(p)	,,
17	(p)	(p)	Mild mosaic
18	(g)	1p	,, (one tuber)
19	–	1p	Healthy
20	1p, 1g	(p)	Crinkle
21	–	(g)	Leaf-roll + interveinal mosaic
22	0	1p	Healthy
23	(1p*w*)	28p	,,
24	1p	(p)	,,
25	1p, 1g	1p	Crinkle (one tuber)
26	1p	1p	Healthy
27	3p	(p)	,,
28	2p	1p	,,
29	1p, 1g	2p	,,
30	0	3p	Mild mosaic (one tuber)
31	(p)	3p, 1g	Healthy
32	1p, 1g	(p)	,,
33	1p	2p	,,
34	1p	1p, 3g	,,
35	3p, 1g	1p, 1g	,,
36	3p	(p)	Crinkle (one tuber)
37	5p	1p	,, (one tuber)
38	0	(p)	Mild mosaic (one tuber)
39	(p)	6p	Healthy
40	1p	0	,,
41	1p	1p	,,
42	2p	4p	,,
43	1p	(p)	,,
44	0	(p)	,,
45	1p	2p	,,
46	0	0	,,
47	3p	(p)	,,
48	(p)	1p	,,
49	2p	3p	,,
50	4p, 1g	(p)	Crinkle (two tubers)
51	0	0	Healthy
52	3p	9p	,,
53	0	(p)	,,
54	0	(p)	,,
55	1p*w*	(p)	,,
56	1p*w*	(p)	,,
57	1p, 1g	(p)	,,
58	(p)	(p)	Leaf-roll

Symbols as for Table I.

infested with *M. persicae*. On 27 June, 75·9 % of the plants above ground were infested with *M. persicae*, and at least 22·2 % with *M. gei*. On 25 July, 91·3 % of the isolated plants carried *M. persicae*, so that the ease with which winged aphides detect and colonize such plants was again very apparent.

The progeny of the experimental plants was collected, stored and planted at the College Farm as described for Centre I, the condition of health being recorded in the last column of Table II. It will be observed that out of fifty-eight isolated plants the progeny of twenty-three (or 39·6 %) proved to be infected with one or more virus diseases. Two of these showed symptoms of leaf-roll alone, two showed leaf-roll and mosaic, eight were infected with a crinkle complex, two with mosaic alone, and eight with a mottling probably representing a very mild infection with a form of mosaic. Here also, as at centre I, there was evidence of considerable spread of virus diseases (in this case mostly of the mosaic type) when only a small crop of potatoes occupied the same field.

Centre III (Hawarden, Flintshire)

At this centre the half-tubers were planted in a mangold crop as at the two previous centres, with the general farm crop of potatoes growing in the same field but distant at least 100 yd. from the nearest experimental plants. No detailed information of the aphis population over a number of years was available, though aphides are common in the surrounding district and the index figure was frequently over 500 of all species per 100 leaves. Nor was there any exact knowledge of the rate of spread of virus diseases though it was believed to be fairly rapid and it is the general custom to make frequent changes in the seed potato stocks grown. A count of the aphis population on the experimental plants was not made, but when the progeny of the forty-eight isolated plants was grown at the College Farm in 1936, eight of them (16·7 %) were shown to have contracted leaf-roll at Hawarden in 1935.

Centre IV (Dyserth, Flintshire)

As at the last centre there was no previous knowledge of the aphis population or of the rate of spread of viruses when this centre was selected. It is, however, only a few miles distant from centre I and with very similar topographical conditions. The aphis population in general is high, and it is a common practice for growers to change their seed potatoes frequently. One important point of difference, however, from

the first three centres described, was the degree of isolation obtained for the experimental plants, these being planted in a crop of sugar beet and at a distance of at least a quarter of a mile from the nearest farm crop of potatoes. This latter crop contained some virus-infected plants, but the percentage was unfortunately not recorded.

When an aphis count was taken on 27 June, ninety-five out of the 100 isolated plants were found to be already infested with *Myzus persicae*. Unfortunately some of the plants were inadvertently removed before lifting time at this centre but the records of the sixty-seven remaining plants, given in Table III, suffice to indicate the nature of the aphis population. Sixty-three of these plants were infested with

Table III

Aphis population on isolated plants and evidence of virus transmission

Centre IV (Dyserth, Flintshire). Isolated tubers grown in sugar-beet crop; no potatoes within a quarter of a mile

Plant	Aphides on 27. vi. 35	Health of progeny 1936	Plant	Aphides on 27. vi. 35	Health of progeny 1936
1	1p	Healthy	35	1p*w*	Healthy
2	3g (p)	,,	36	2p, 1g	,,
3	(p)	,,	37	3p	,,
4	(p)	,,	38	(1p*w*)	,,
5	2p*w*, 8p	,,	39	(p)	,,
6	1p	,,	40	7p	,,
7	1p*w*, 3p	,,	41	1g	,,
8	1p	,,	42	1p*w*, 10p	,,
9	(p)	,,	43	3g, (p)	,,
10	1p	Leaf-roll	44	3p*w*, 18p, 2g	,,
11	1p*w*, 1g	Healthy	45	2p, 1g	,,
12	1p	Leaf-roll	46	(g)	,,
13	(p)	Healthy	47	4p	,,
14	0	,,	48	3p	,,
15	2p*w*, 1p	,,	49	10p	,,
16	1p	,,	50	3p, 6g	,,
17	4p, 1g	,,	51	8p, 7g	,,
18	(p)	,,	52	8p, 4g	,,
19	1p	,,	53	4p	,,
20	(p, g)	,,	54	15p, 1g	,,
21	(p)	,,	55	(p)	,,
22	2g (p)	,,	56	3p	Leaf-roll
23	1g	,,	57	4p	Healthy
24	1p	Leaf-roll	58	2p, 2g	,,
25	1p	Healthy	59	5p	,,
26	1p, 1g	,,	60	4p, 2g	,,
27	1g (p)	,,	61	1p*w*, 8p	,,
28	(1p*w*)	,,	62	3p	,,
29	5p, 2g	,,	63	4p, 1g	,,
30	6p, 1g	Leaf-roll	64	2p, 1g	,,
31	1p*w*, 7p, 1g	Healthy	65	1p	,,
32	4p, 4g	,,	66	(p)	,,
33	3p, 2g	,,	67	1p*w*, 2p	Mosaic
34	2p, 2g	,,			

Symbols as for Table I.

M. persicae on 27 June and twelve *winged* forms were seen although only three leaves per plant were examined; twenty-seven plants at least were infested with *Macrosiphum gei*. These figures present ample evidence to show that when potatoes are planted singly, in isolation in a root crop, and with no other potatoes within a distance of a quarter of a mile, winged aphides have no difficulty in detecting and colonizing the single unit plants.

When the health of the progeny is examined in the record shown in Table III, it is clear that the spread of viruses was much less than at the first three centres. All four centres were located in districts definitely regarded as unsuitable for seed-potato production by reason of the rapid spread of viruses, and the data now presented give some indication of a correlation between virus spread in such localities and the distance between potato crops. It is evident also that in such districts it will be difficult to secure a degree of isolation sufficient to ensure the maintenance of health in seed stocks. At centre IV, although the isolation was better than could ordinarily be obtained, and notwithstanding that aphis infestation was low during 1935 in this district, six out of the sixty-seven isolated plants (8·9 %) had contracted virus diseases (five having leaf-roll, and one showing mosaic) in that year.

Centre V (College Farm, near Bangor, Caernarvonshire)

This centre is in an entirely different district from those described above. It is more humid and low-lying; being exposed to the open sea one mile to the north and backed by mountains within a mile to the south. The aphis population at this centre is always much lower than in the eastern districts where the first four centres are located, but in certain seasons the population is fairly high with an index figure of about 300 aphides per 100 leaves examined. A museum of virus infected potatoes is maintained each year in the potato field and the rate of spread of viruses is appreciable, though not so rapid as in the eastern districts to which reference has been made. The district can be regarded as intermediate between east and west both as regards aphis population and rate of virus spread.

The experimental half-tubers were planted in isolation in a crop of mangolds, about 40 yd. to the east of the general farm crop of potatoes and the virus museum; all being in the same field. The mangold crop with its isolated potato plants was on a steep slope with an easterly, exposed aspect. The records of aphis infestation and the spread of disease as shown in the progeny are given in Table IV. In addition,

since there was a heavy attack of the capsid *Calocoris norwegicus* on the first row (plants 1–33), an asterisk indicates severe damage to the plant.

Table IV

Aphis population on isolated plants and evidence of virus transmission

Centre V (College Farm near Bangor, Caernarvonshire). Isolated plants in mangold crop and in same field as heavily infected potatoes

Plant	Aphides present on three leaves on 21. vi. 35	2. vii. 35	25. vii. 35	Health of progeny 1936
1	0	0	1p (par)	Healthy
2	0	3p	1p	,,
3	0	1p*	0	,,
4	0	2p*	0	,,
5	0	(p)*	0	,,
6	0	0*	0	,,
7	0	0	0	,,
8	0	1p*	1p (par)	,,
9	1p	0*	0	,,
10	(p)	(p)*	1p	,,
11	(p)	0*	0	,,
12	0	1p*w**	0	,,
13	0	2p	0	,,
14	1p	1p*	0	,,
15	(p)	0*	0	,,
16	0	(p)	0	,,
17	0	0*	0	,,
18	0	0*	0	,,
19	(p)	1p*	0	,,
20	0	0*	0	,,
21	0	1p*w**	0	,,
22	(p)	0*	1p (par)	,,
23	0	0*	0	,,
24	0	1g*	0	,,
25	0	0*	1p	,,
26	0	1g*	0	,,
27	0	0*	0	,,
28	0	0*	0	,,
29	0	0*	0	,,
30	0	0*	0	,,
31	1p	0*	0	,,
32	0	0*	0	,,
33	0	0*	0	,,
34	(p)	(p)		,,
35	0	0		,,
36	0	1p		,,
37	(p)	(p)		,,
38	0	0	–	,,
39	0	0	–	,,
40	0	1p (par)	–	,,
42	0	0	–	,,
43	0	0	-	,,
44	0	0	–	,,
45	0	1p	–	,,
46	1p*w*	18p		,,
47	0	1p	–	,,

* = plant severely damaged by *Calocoris norwegicus*. (par) = parasitized. Other symbols as in Table I.

Table IV (*cont.*)

Plant	Aphides present on three leaves on 21. vi. 35	2. vii. 35	25. vii. 35	Health of progeny 1936
48	0	0	–	Healthy
49	1p	3p	–	,,
50	1p	1p	–	,,
51	0	0	–	,,
52	1*gw*	1g (par)	–	,,
53	1p	2p		,,
54	(p)	4p	–	,,
55	0	0	–	,,
56	0	1p	–	,,
57	0	0	–	,,
58	0	0	–	,,
59	0	2p	–	,,
60	1p	0		,,
61	0	1p	–	Leaf-roll
62	0	0		Healthy
63	0	0	–	,,

The aphis infestation of the isolated plants was markedly lower than at the centres previously described although thirty-four out of the sixty-three plants had *Myzus persicae* present at one or other date of examination. This lower infestation was due to the fact that there was only a negligible migration of winged aphides, which was confirmed by the data obtained in collecting winged aphides daily in the nearby mechanical trap (Davies, 1935). The spread of virus diseases to these isolated plants was correspondingly slight, for only one plant yielded infected progeny; a result which was a little surprising in view of the large number of diseased plants growing in the same field. Other experiments in the same field confirmed the view that the lack of virus transmission was due to scarcity of winged migrants. A small plot of 100 healthy potatoes was grown in isolation within the mangold crop, primarily for the purpose of studying the aphis population and as a control for spraying trials The aphis population on this unsprayed, control plot proved to be very slight, not exceeding ten per 100 leaves, but it was decided to take a sample of the tubers for planting in 1936 in order to discover the amount of virus transmission occurring under these conditions. This was compared with a plot of similar size growing in the general crop of potatoes alongside the virus museum. The progeny of the isolated plot in the mangold crop showed 1·1 % infection only; indicating a slight migration of winged forms to the crop, for only such forms had access to the plants. On the other hand, 10·74 % of the plants adjacent to the virus-infected material became infected, this representing disease transmission to be ascribed both to Alatae and Apterae. There

was no evidence that *Calocoris norwegicus* was a vector of virus diseases under field conditions.

Centre VI (Madryn, Bodfean, Caernarvonshire)

This centre, and the following one, were selected in the districts where there had been no increase in virus infection among potato stocks since they were introduced in 1928. At each centre fifty half-tubers were planted in isolation in mangold crops with the general farm crop about 40 yd. distant in the same field. In view of the fact that the progeny of the general crops had showed no increase in virus infection during 8 years, it was only necessary to seek further confirmation of a direct correlation between this lack of transmission and the number of winged aphides present. It was only possible to make a single aphis count, but this sufficiently indicates the slight nature of the infestation, although the sheltered position of this particular field would permit greater migration than is general in this district. The progeny from half of the isolated plants only was removed to the College Farm for subsequent planting, and it will be seen from Table V that no disease occurred in this progeny in 1936. This failure to transmit was not due to an entire absence of infected plants in the main crop, for, from some unexplained cause, the infection in this crop increased from the normal 0·5 to 1·15 % in 1935.

Table V

Aphis population on isolated plants and evidence of virus transmission

Centre VI (Madryn, Bodfean, Caernarvonshire). Isolated plants in mangold crop. Two acres of farm crop of potatoes in same field. Progeny taken from only twenty-five plants

Plant	Aphides on 10. vii. 35	Health of progeny 1936	Plant	Aphides on 10. vii. 35	Health of progeny 1936
1	0	Healthy	14	0	Healthy
2	1*pw*	,,	15	0	,,
3	0	,,	16	0	,,
4	0	,,	17	0	,,
5	2p	,,	18	1g	,,
6	0	,,	19	1p	,,
7	(1*pw*)	,,	20	0	,,
8	0	,,	21	0	,,
9	0	,,	22	1p	,,
10	2p	,,	23	0	,,
11	0	,,	24	(p)	,,
12	2p	,,	25	0	,,
13	1*pw*	,,			

Symbols as for Table I.

Centre VII (Hendre, Aberdaron, Caernarvonshire)

This centre is one of the best seed-producing farms; it is low-lying, exposed, and stocks have been grown without increase of virus infection since 1928, the figure being 0·52% in 1935. The aphis population has always been very low and the index figure of *M. persicae* in mid-July has not, normally, exceeded twenty per 100 leaves. This index figure since 1928 has been as follows: 1928 (negligible, all spp.), 1929 (24, all spp.), 1930 (5, *M. persicae*), 1931 (58, *M. persicae*), 1932 (44, *M. persicae*), 1933 (82, *M. persicae*), 1934 (26, *M. persicae*), 1935 (12, *M. persicae*).

It will be seen from Table VI that the aphis infestation of the isolated plants at this centre was very slight and that there was no evidence of transmission of any virus having occurred in 1935, as judged by the state of health of the plants grown from four tubers taken from each of the plants in that year.

Table VI

Aphis population on isolated plants and evidence of virus transmission

Centre VII (Hendre, Aberdaron, Caernarvonshire). Isolated plants in mangold crop. Farm crop of potatoes in same field of two acres. Progeny taken from only twenty-five plants

Plant	Aphides on 10. vii. 35	Health of progeny 1936	Plant	Aphides on 10. vii. 35	Health of progeny 1936
1	0	Healthy	14	0	Healthy
2	2p	,,	15	0	,,
3	0	,,	16	0	,,
4	0	,,	17	1p, 1g	,,
5	0	,,	18	1p	,,
6	0	,,	19	0	,,
7	1p	,,	20	0	,,
8	0	,,	21	0	,,
9	0	,,	22	0	,,
10	0	,,	23	1*pw*	,,
11	0	,,	24	0	,,
12	1g	,,	25	0	,,
13	0	,,			

Symbols as for Table I.

DEVELOPMENT OF THE APHIS POPULATION ON ISOLATED PLANTS COMPARED WITH THAT ON THE NEAREST POTATO CROP

The development of the aphis population on isolated plants and on a general crop of potatoes can best be compared by studying the data at centre I (Berthymaen), presented in Table VII.

All the isolated plants infested with *M. persicae* on 14 June were still infested with this species on 28 June (cf. Table I), and even on 26 July 69·5% of those initially infested still had this species present.

This indicates that colonization was successful following the initial migration of the winged migrants.

Table VII

Infestation of aphides on isolated plants and (in brackets) on the general farm crop of potatoes at Berthymaen, 1935

Date	14 June	28 June	26 July	25 August
No. of plants	75*	98 (50)	98 (50)	99 (50)
Percentage infested with *M. persicae*	62*	98·9 (98)	63·6 (90)	25 ( )*
No. of *M. persicae* per 100 leaves	20·8*	164 (294)	30·3 (210)	5·9 (79·2)
No. of winged *M. persicae* per 100 leaves	8·4*	7·8 (4·6)	0·0 (0·0)	0·0 (0·0)
No. of *M. gei* per 100 leaves	1·3*	22·3 (122)	13·4 (232)	3·8 (89·6)
No. of winged *M. gei* per 100 leaves	0·0*	1·02 (1·3)	0·0 (0·0)	0·0 (0·0)

* No aphis count made on general farm crop

The increase in the population of *M. persicae* was quite rapid since within a fortnight the index figure had increased from 20·8 to 164 per 100 leaves. But there was also a rapid decline (cf. Table VII), since by 26 July the index figure had fallen to 30·3, and on 25 August it was only 5·9 for *M. persicae* per 100 leaves. The continuous observation maintained on these plants at Berthymaen clearly showed that this reduction was due to the activities of predators and parasites which, with the limited environment of the isolated plant, took a heavy toll of the aphides. There was evidence also that thunder-rain reduced the population on the exposed isolated plants. The rapid increase in the total population of *M. persicae* and its subsequent decline was also evident at the other centres.

A comparison with the aphis population on the general potato crop in the same field shows the latter to be higher, the index figure on 28 June being 294 *M. persicae* compared with the 164 of the same species on the isolated plants. Further, the infestation on the general crop was better maintained, for on 26 July the index figure in the crop was still 210 compared with the 30·3 *M. persicae* per 100 leaves of the isolated plants. From this date, however, the population declined rapidly until only 79·2 *M. persicae* could be counted per 100 leaves on 25 August.

The period during which winged aphides were collected on the isolated plants is of some interest, since it is believed to indicate the time during which the initial migrants were arriving and actively moving from plant to plant. At three centres a sequence of records was obtained of the winged forms taken on the isolated plants. The average number per plant was as follows: Centre I: 14 June, 8·4; 28 June, 7·8; 26 July, nil; 25 August, nil. Centre II: 27 June, 2·5; 25 July, nil. Centre V:

21 June, 0·5; 2 July, 1·0; 25 July, nil. This arrival of winged migrants on potatoes during June and the decline in numbers at the end of July has been noted for several seasons. It is not until after this period that winged forms, which have been produced on the potato crops, begin to appear among the aphis colonies.

The infestation of *Macrosiphum gei*, although slight, presented points of some interest. It was never high on the isolated plants at any centre, and, even at centre I, was only 22·3 per 100 leaves on 26 July, declining to 3·8 by 25 August. In the general farm crop of potatoes in the same field, however, the corresponding index figures of population were: 122 on 28 June, 232 on 26 July, and 89·6 on 25 August. This lower infestation of *M. gei* on the isolated plants as compared with that of the general farm crop was also apparent at centres II and IV; the index figures on the isolated plants at these centres being 2·8 and 25·3 per 100 leaves respectively, whereas in the general crop the figures on the same day were 120 and 70 per 100 leaves respectively.

DISCUSSION

(a) *Colonization*

The remarkable ease with which winged *Myzus persicae* detect and successfully colonize isolated potato plants has been clearly demonstrated; for instance, at centres I, II, and IV, 98·9, 91·3, and 94·0%, respectively, of these plants were visited by winged migrants of this species before the plants were 6 in. high. Since this infestation took place in a season when the aphis population in these districts was unusually low, it can be inferred that *M. persicae* will migrate to and populate any potato crops growing even in relative isolation in these districts.

The distance over which winged aphides will migrate is difficult to discover because there is no easy way by which marked winged aphides can be attracted after release. There is some evidence (cf. Davies, 1936) that winged aphides can be carried involuntarily over considerable distances, but this enforced *transportation* should not be confused with *voluntary migration*, which is the type of migration associated with the normal colonization of host plants. The records of infestation by winged aphides of the exceptionally isolated plants at centre IV are, therefore, particularly interesting in this connexion. At this centre the isolated plants, growing in a sugar-beet crop, were distant at least a quarter of a mile from the nearest potatoes; thus fixing this distance as the minimum flight of the virulent migrants. The root field, moreover, was on a plateau

on the reverse slope from the nearest potato crop and facing the direction from which the light, dry breezes usually waft the migrating aphides. It is probable, therefore, that the migrants had covered a distance much greater than the quarter of a mile separating the isolated plants from the nearest potato crop.

(*b*) *Spread of virus infection*

The contrast in the aphis population of potato crops in the eastern districts where spread of viruses is usually rapid, with that of western districts where little if any spread occurs, has been fully established (Whitehead *et al.* 1932; Davies, 1934). It was, however, uncertain whether this was due to a difference in numbers of winged migrants or to a difference in the rate of reproduction of aphides within the crop. The present work shows that there was a very much smaller number of winged migrants at the western centres than at those in the east, and that the possibility of virus transmission must be correspondingly reduced in the west. Investigation carried out by the writers in 1934 (Davies & Whitehead, 1935) had shown that only a minute percentage of winged aphides (i.e. 0·34 %) were already infected with viruses when they arrived on the experimental potato plot which, in point of fact, was located at centre I. This was confirmed in 1935, although only 200 winged *M. persicae* could be collected during a daily search from 15 June to 23 July at this centre, in what proved to be an exceptionally low year of infestation. The winged migrants so collected were despatched to Bangor and isolated in batches of five each on healthy half-tubers. Although the numbers were small the results were in keeping with those of 1934 for none of the aphides proved to be infected with a virus. It is interesting, however, to note that of twenty-nine winged *M. persicae* taken from "ground keepers" on 14 June at least two were infected with leaf-roll. It is important to remember that this virtual freedom from virus infection of the initial migrants to potatoes was demonstrated at a centre where virus infection in a crop had increased in *two years* from 0·23 to 33·0 % (Whitehead *et al.* 1932). It would seem, therefore, that this rapid spread of viruses must have been due to aphis movements within the crop itself, but whether by winged forms or Apterae was not determined. The present results, by giving the amount of transmission to isolated plants, implicate winged forms only, and the high percentage of plants infected suggests that transmission was due to short, frequent movements of winged aphides within the crop. At centre I, 94·6 % of the plants became infected, and even at centre II, with only a small plot

of potatoes in the field, infection was carried to 39·7 % of the isolated plants. At centre III this percentage fell to 16·7, due, it is presumed, to the greater distance (100 yd.) from which infection had to be carried. These facts confirm the opinion of the writers that the spread of virus diseases from crop to crop is due mainly to the relatively small numbers of winged forms still migrating after feeding on many potatoes, or to winged aphides, again usually fairly small in numbers, produced later in the season on potato crops.

(c) *Protection from virus infection*

The seven centres at which the present work was carried out fall into three reasonably distinct groups. The first group consists of centres VI and VII where both winged aphides and sources of virus infection were exceptionally small in number. Under such conditions the problem of protection is simple and need only be such that Apterae are prevented from crawling from plant to plant. The second group is represented only by centre V, where sources of infection are extremely numerous and the possibility of adequately protecting a crop from infection will be determined by seasonal factors which regulate the alate aphis population. In the year under consideration (1935) very low counts of Alatae were made throughout North Wales and, at centre V, the number had steadily declined from a "peak" infestation in 1933. This low alate population was reflected in the small amount of virus transmission (i.e. 1·6 %) which occurred at centre V, notwithstanding the numerous sources of infection within 40 yd. of the isolated plants. Even a small, compact plot of potatoes at a similar distance from infectors was only infected to the extent of 1·1 %, whereas a plot adjacent to infectors, and so accessible to crawling Apterae, had disease transmitted to nearly 11 % of the plants. In a year unfavourable for the development of aphides, therefore, the proximity of heavily infected potato stocks had little effect upon the spread of virus diseases, and a fairly high degree of protection was afforded by a distance of 40 yd. from infectors. On the other hand, in seasons favourable for aphis development (as in 1933) the population at centre V approximates to that found in 1935 at centres I–IV, and would be included, with them, in the third group, in which Alatae are numerous and sources of infection are not negligible. Under such conditions a distance of 40 yd. from infected plants gives little or no protection to the isolated potatoes. It is at centre IV, however, that the difficulty of adequately protecting a crop becomes most apparent. Here, relatively large numbers of winged aphides were in movement and disease was

transmitted to practically 9% of the isolated plants. The latter were at a distance of a quarter of a mile from the nearest potato crop, and both wind direction and topographical features, as well as the unusually low aphis population (for that centre) operated against any great amount of transmission of disease. Moreover, it will be realized that the likelihood of infected winged aphides passing from plant to plant was probably less than would have been the case with adjacent plants of a general crop, whilst wingless forms, produced on an infected isolated plant would have little chance of transferring the infection to other plants in isolation.

It can safely be said that any practicable means of protection of a healthy crop of potatoes, based on distance from a partially infected stock, would fail under conditions approximating to those found at centres I–IV, and that effective protection would be still more difficult in arable areas where potatoes are an important crop in the rotation. These facts are of particular importance in framing regulations for the improvement of the health of seed potato stocks. The present work shows that it is not enough merely to require minimum distances of isolation between stocks, without taking cognisance of aphis population or of the state of health of neighbouring crops. The criterion should obviously be the risk of infection of the stock seeking certification, and this is not solely, or even mainly, a question of distance. What, in the opinion of the writers, should be aimed at is the segregation of districts (or farms) into classes based on a survey of all these factors, with appropriate conditions for certification of seed stocks in each case; some such scheme as this was suggested by the writers and Mr J. F. Currie in 1932 (Whitehead *et al.* 1932). Uniformity of regulations over large areas of country is especially to be deprecated, for the distance of isolation required will be unnecessarily great in really good seed producing districts and entirely ineffective elsewhere.

Summary

1. The investigations were planned to ascertain the extent of the migrations of winged aphides, their ability to detect individual plants, the development of aphis population following colonization, and the extent to which virus diseases may be transmitted to isolated plants by winged aphides.

2. Plants were isolated in root crops on seven farms and at varying distances from probable sources of virus infection. Four farms were located in districts where aphis population is usually high and viruses spread very rapidly; two in districts where both aphides and spread of

viruses are minimal, whilst one farm could be regarded as intermediate in both respects.

3. Evidence is submitted to show that winged aphides have no difficulty in detecting and colonizing isolated plants. Their numbers were large at the four eastern centres and very small at the three western centres. In all cases the aphis population on isolated plants was less than that of the nearest general crop and suffered more from predators and parasites.

4. It is shown that isolated plants can be reached by migrants from a distance of at least a quarter of a mile, and probably much further. Additional evidence is given that these initial migrants introduce little virus infection to potato crops from extraneous sources. The importance, however, of the later movements of winged aphides, whether initial migrants or those subsequently produced on potatoes, in spreading viruses from crop to crop, is emphasized.

5. The practical aspects of protecting potato stocks is discussed under conditions of (*a*) heavy infestation by winged aphides and proximity of partially diseased crops; (*b*) minimal winged aphis infestation where numerous sources of virus infection occur in neighbouring crops; and (*c*) where both aphis infestation and sources of infection are minimal. It is considered that regulations for the improvement of health in seed-potato stocks should take cognisance of these various possibilities.

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FACTORS AFFECTING THE FLUCTUATIONS IN THE POPULATION OF *TOXOPTERA AURANTII* BOY. IN PALESTINE

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(With 4 Text-figures)

Introduction

Aphids become very troublesome in Palestine only on occasions when weather conditions have been exceptionally favourable for their development and reproduction. Otherwise they are of little importance to the farmers. Thus *Toxoptera aurantii* Boy. is seldom found in the *Citrus* grove throughout the summer and is scarce in the fall and winter. It becomes numerous only in the spring for a short time, and, as a rule, the damage caused is of little importance. Occasionally, however, without warning, an unexpectedly serious infestation takes place, when the farmers become alarmed and seek to employ methods of control. But the control measures are thought of when it is too late—after the damage has been done, when spraying brings no benefit and, on the contrary, may harm the trees. For these reasons it was found to be of interest to study the factors underlying such sporadic outbreaks and to analyse them with a view to forecasting possible infestations. The following is an attempt to analyse some of the factors controlling the increase and decrease in population of the *Citrus* aphis.

Forms of the aphis

Like many other aphids in Palestine, *Toxoptera aurantii* Boy. can reproduce continuously throughout the year by viviparous parthenogenesis, no sexual reproduction taking place. Although the insect was bred for over 3 years no sexual, egg-laying females were obtained in the broods nor were any discovered in the grove. The forms most prevalent are the agamic apterous and agamic alate females. The ratio between the numbers of the two forms depends upon external factors as well as food conditions, and a more extended discussion of this subject is given

elsewhere (Rivnay, 1937). An additional alate form was discovered in the course of breeding. This is the male of the species, perhaps a vestigial member of the sexual forms.

Rate of development

The data presented below were obtained by breeding the species in the laboratory.

The individual was bred on a soft twig, one end of which was placed in water and the other end in a short test-tube. The development of the

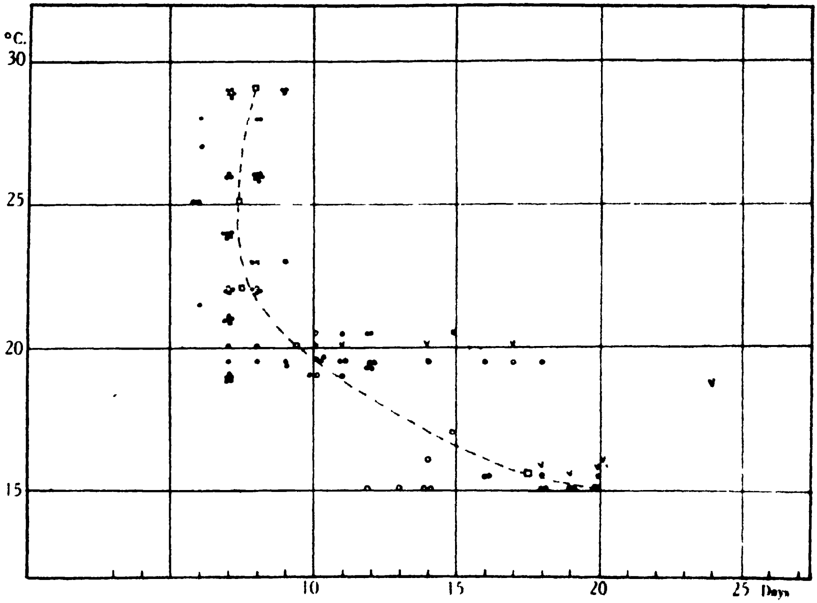

Fig. 1. Curve presenting relationship between temperature and rate of development of the aphis *Toxoptera aurantii*. Each dot represents average period of development of a few apterous females; the wedge represents that of a few males.

apterous agamic female from its birth until it gives birth lasts, under favourable conditions of temperature, about 6–7 days. Fig. 1 presents the rate of development of the apterous agamic female at various degrees of temperature, each dot representing the average record of one breeding, which consisted of three or five females. It is noticeable that the temperature at which development is shortest is between 22 and 25° C. This temperature proved to be the most favourable also for reproduction. At 18–22° C. development is not much slower than at the optimum

temperature. The development may then be from 7 to 12 days, the average being a period of 9½ days. This has a great bearing as a factor in the problem of infestation. At the temperature of 15° C. the development of the aphid takes about 2–3 weeks, and below this temperature the development is so slow that it is of little importance as a factor of infestation. If the temperature rises above the optimum of 22–25° C., development is slightly retarded (see Fig. 1).

REPRODUCTION

The female aphis begins to reproduce almost immediately after she has reached the adult stage and continues to bear young until 2 or 3 days before she dies. During the course of this time a peak in the curve

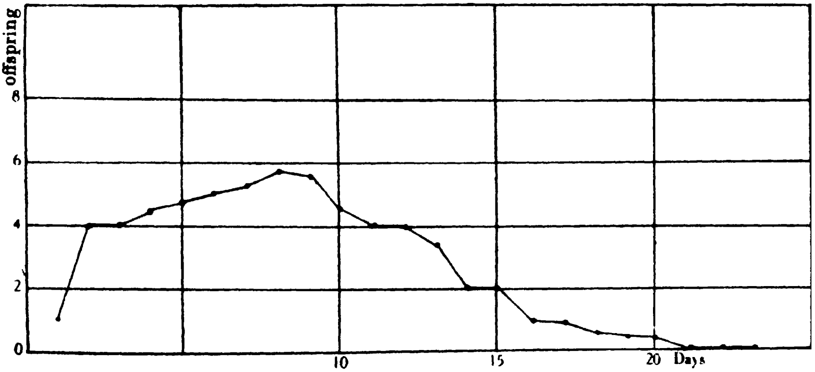

Fig. 2. Curve representing the rate of reproduction of the *Citrus* aphis *Toxoptera aurantii* at a temperature of 22° C.

(Fig. 2) which presents the rate of reproduction may be distinguished, covering a short period when the rate of reproduction of this particular female is at its maximum. After this the rate of reproduction decreases gradually until only one nymph is born in approximately every 2 days. The curve in Fig. 2 represents the rate of reproduction based on the average taken from ten individuals at a temperature of 22° C. It is noticeable that at this temperature the maximum number of young that one female bore in 1 day was approximately five to six. However, in the course of the several breedings some individuals bore even seven to eight young in 1 day. The rate of reproduction decreases as the temperature falls. Thus, at a temperature of from 10 to 13° C., one nymph was borne by one female each day. No reproduction took place at 7° C. At

a temperature above the optimum, reproduction diminishes, so that at a temperature of 33° C. individuals do not reproduce or else they bear only very few young, approximately one nymph a day during their short life. At a temperature of 34–35° C. no young were born at all.

When the temperature and humidity are favourable one female may bear as many as eighty offspring during her lifetime. On the average the maximum production of one female is about seventy young, which takes place at the optimum temperature of 22–25° C. With decrease or increase of the temperature the reproductive power of the female also decreases (see Table I).

Table I

Temp. in ° C. ...	12·5–14·5	15–18	22	25	28–29	32–33	34–35
No. of females	6	11	11	5	11	4	50
Total no. of their offspring	66	302	656	334	274	29	0
No. of offspring per female	11	27	60	67	25	7	0

Effects of temperature upon mortality

Under favourable conditions and optimum temperature, the apterous female of the *Citrus* aphis may live from 20 to 30 days. In the winter, however, at an average temperature of about 15° C., her life may be prolonged to about 60 days. Temperatures above 25° C. shorten her life to a great extent. Thus, at 28–29° C., the average life of ten individuals was about 10·3, while at 32–35° C. the average length of life was about 6 days; of fifty females, twenty-one died within 3 days, twenty-four within 4–9 days, and only six survived from 10 to 11 days. At 36° C. most of fifty insects died within a day or a fraction of a day, few surviving to the next day and then dying.

Young nymphs of the first and second instars can hardly survive above 30° C. For instance, out of 121 young reared in four breedings at a temperature of 30–32° C., only four individuals survived to reach the adult stage. These were much smaller than the normal size of the species and died within 2 or 3 days, leaving no offspring.

Effects of humidity

Generally the aphids were bred under conditions of high relative humidity. Some breedings, however, were carried out in very dry environments, and it was found that this insect is quite tolerant to external changes of relative humidity as long as it is kept within optimum ranges of temperature and a good supply of food is available. A very low relative humidity may stimulate the development of wings, but

otherwise only slight effects were noticed on the rate of development and reproduction. However, at higher temperatures it was found that a low relative humidity greatly affects the insect and lowers the thermal death-point.

ANNUAL FLUCTUATIONS IN THE POPULATION OF THE APHIS

The annual fluctuations of the population of the black *Citrus* aphis throughout the year are characterized by a distinct increase towards the end of February and March followed by a sudden decrease in April. This decrease may be so abrupt that not a single living aphid can be found on the trees 10 days after the climax had taken place. Slight reinfestations may appear during the summer months if the weather is exceptionally mild; otherwise, no aphids are to be found until the following fall.

The infestation of the spring 1936, which was quite troublesome, was carefully studied in the groves of the Experiment Station at Rehovoth and is described below. From several reports it is evident that the situation throughout the country was similar to this, and it may be considered as typical of the general situation along the coast of Palestine.

Single colonies of aphids were observed throughout November, December and early January. Towards the end of January and early February they began to be more common, and towards the end of February the colonies increased to such a degree that the insect became a nuisance. Towards the middle of March the pest presented a problem, and, unlike preceding years, *Citrus* growers were making preparations to employ measures of control. However, towards the middle of April the insect diminished very considerably, and about 25 April not a single living aphid was to be found on the trees.

Counts, whereby the annual fluctuations could be presented, were not made for the following reasons: The number of aphids per tree could not give a true picture of the infestation because one single twig on a tree may harbour more insects than another tree where the aphids are distributed on every new shoot. Yet the latter presents a more serious infestation. Again, the counting of infested trees would not give a true picture of the situation because a single small colony on a tree would classify the tree as infested and it would not distinguish it from a tree which was really heavily infested with the pest. It was found more advisable, therefore, to present the curve of fluctuation in terms of four degrees of infestation, namely, (1) no infestation, (2) slight infestation, where single colonies were present here and there, (3) moderate infestation,

where the pest became more conspicuous on a large percentage of trees, and (4) heavy infestation, when every tree had every new shoot infested. In these terms the fluctuation of the aphid population in the spring of 1936 is shown in Fig. 3.

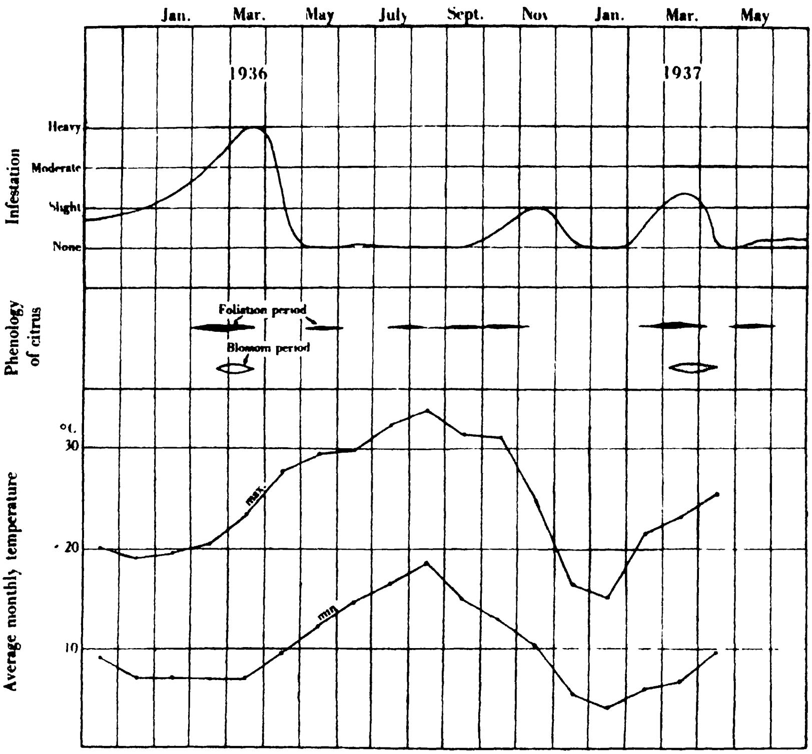

Fig. 3. Graph showing the fluctuation in the population of *Toxoptera aurantii* during the years 1936 and 1937, together with the phenology of *Citrus* trees and the fluctuations in the temperature of the same period in Palestine.

Food as a factor in the increase of population of the aphid

One of the factors responsible for the curves in Fig. 3 is the availability of the food. *Toxoptera aurantii* feeds on very soft, young leaves or on the tips of newly developed shoots. As soon as the leaves become mature and hardened, the aphids leave them. In laboratory experiments, several young nymphs were placed to feed upon mature leaves, but the young crawled away from these leaves in search of better food. When they were

confined to such food they starved within a day or two. On the other hand, when placed upon a soft twig, they always remained and developed thereon. In view of this fact it is quite obvious that the abundance of the aphids depends to a great extent upon the abundance of suitable food, that is, new growth. For this reason the peak of the aphis infestation takes place simultaneously with the new growth of the spring, as may be observed in Fig. 3, i.e. during the month of March. Infestations at other periods of new growth, during the summer, are hindered by unfavourable conditions of temperature as will be discussed below, while the new growth during the months of September and October stimulates and encourages the reinfestation of the grove after the summer.

TEMPERATURE AS A FACTOR INFLUENCING ANNUAL FLUCTUATIONS OF THE APHIS

The temperature during the months of December and January is quite low and insufficient to make possible a great multiplication of the aphis. On the one hand, the food supply during these months is quite scanty and discourages the mass reproduction of the aphis and on the other hand, the temperature itself is unfavourable for its development. The temperature during the nights is far below that at which development and reproduction can take place (below 8–10° C.) and the day temperature is on the average from 12–15° C. At such a temperature the development of the aphis is about three weeks and consequently reproduction is greatly retarded. Thus, a noticeable increase of this insect cannot take place during December, January and early February.

As mentioned above, the optimum temperature for development and reproduction of the aphis is between 22–25° C. As a rule the average day temperature during the month of March in Palestine is within these temperature limits. It is true the nights are still cool, but the temperature during the day is sufficiently high to enable the aphis to produce at least three generations during the month, so that one single individual is capable of giving rise to over 100,000 offspring within the month of March. Hence the sudden increase of the insect during that month. However, this progress is checked during April, the temperature of which is catastrophic for the insect. It has already been stated that at 32° C. the insect is no longer capable of development; at 33–34° C. it no longer reproduces; and at 36° C. it dies within a short period. If this high temperature is coupled with very low atmospheric humidity, death takes place even faster or instantaneously. During April it happens, quite often, that the temperature rises even above this limit. Thus, during

April 1936, on the 12th and 18th days of the month, the maximum temperature in Rehovoth reached 41° C. and the relative humidity fell to 10%; during the 21st to 24th of the same month the temperature was 41–42° C. Inspection in the grove showed that a great percentage of aphids died after the 18th yet a few were still alive, the high temperature apparently not lasting long enough to kill all. However, not a single living aphis was found on the trees after the 25th. No doubt, as a result of the dryness of the atmosphere, many of the young aphids developed into alatae, and when the detrimental temperature set in they were capable of flying off the trees in search of cooler places in the ground. All the apterous forms were no doubt killed, thus leaving no individuals on the trees. The general maximum temperature of that summer in Palestine was too high (33° C.) to allow a re-establishment of the aphis. In fact, throughout the summer months the *Citrus* grove adjacent to the Experiment Station was free from infestation. The first colony of reinfestation was found on 7 October 1936, and consisted of about six mature apterous females and their offspring, the oldest of which were about 4–5 days old. The mature aphids were no doubt borne by an alate individual which emerged from the ground after the weather had cooled somewhat, probably towards the end of September.

Limiting factors

Since it has been pointed out how the annual fluctuations of the temperature bring about the annual fluctuations of the aphis population, it is of interest to know what caused the epidemic outbreaks of this insect in certain years. In 1934 and 1935, for instance, the infestation of the aphids in the grove in Rehovoth was normal; it became exceptionally heavy during the spring of 1936, and was very slight during the spring of 1937. What factors lie behind these degrees of infestation? The question of availability of food does not enter into consideration now, since the *Citrus* trees develop new growth every year. The cause must lie in the weather conditions of each year, which are variable. For comparison let us take the two extreme infestations, namely, that of the spring of 1936 and of the spring of 1937. As mentioned above, the infestation of 1936 was noticed as early as November-December of the previous year. This infestation increased gradually without any cessation during January 1936, and towards the end of February of the same year it was heavy. Such was not the case the following year.

The early colonies that appeared in October continued to develop throughout that month and during early November. Towards the end

of this month they disappeared and were not to be found in the grove during December of that year nor during the following month. New small colonies appeared only in February and lasted throughout March but disappeared during the early part of April. Single colonies were seen throughout the summer, the weather having been exceptionally mild during that time. The curve of this infestation, based on the same scale as that of 1936, is presented in Fig. 3.

The chief difference between the two curves is that the curve of 1935–6 rises gradually and continuously during December and January, whereas that of 1936–7 descends noticeably during these two months. This descent is responsible for the low peak during March of that year. The period from February to early April was too brief for the insects to multiply to such an extent as to cause a heavy infestation. In the previous year the species continued to develop uninterruptedly from November to March, and this factor was responsible for the high peak in March. If we compare the temperature of December and January in the two seasons, the cause of such a picture is quite obvious. While the winter of 1935–6 was mild, that of 1936–7 was severe (see Fig. 4). In the latter the daily maximum temperature was, as a rule, below the line of 18° C., while in 1935–6 the maximum temperature was generally above that line. As mentioned in the foregoing, the development of aphids at a temperature below 18° C. is quite slight and of long duration. Reproduction is also more rapid above the line of 18° C. than it is below the line. In addition, the winter of 1936–7 was accompanied by heavy, dry Eastern desert winds, and during the nights the temperature fell to freezing point while the humidity was quite low. The previous winter, on the contrary, was quite mild.

THE ECONOMIC STATUS OF THE INSECT

As a rule the aphis causes damage to young groves only because there is always an abundance of new growth in young trees. However, the damage is not very serious since the development of the trees in the summer months is sufficient to make up for the loss suffered during February-March.

More serious damage may result when a heavy attack occurs in a mature grove at the time of blossom. The feeding of the pest on blossom shoots causes the blossom to drop prematurely. Reports of damage of this kind were received particularly in the spring of 1936 from Petah Tikvah, Rehovoth and Ness Ziona.

Blossoming occurs, as a rule, after the peak of the infestation has been attained, i.e. when the aphid population is on the decline. The

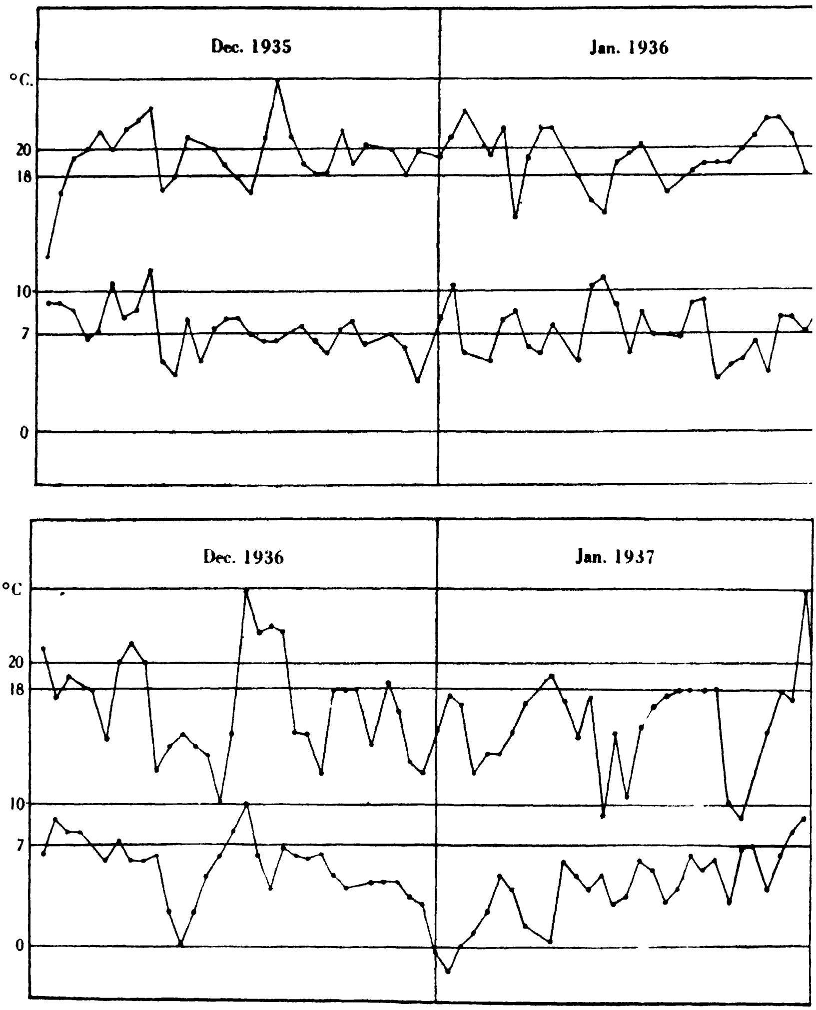

Fig. 4. Daily fluctuations in the temperatures of December and January in 1935–36 and 1936–37.

damage then cannot be considered serious. In 1936, because of the mild winter, spring foliation, as well as blossoming, occurred a few weeks

earlier than the normal time. Fig. 3 shows the difference in these phenomena as they occurred in 1936 and 1937 in Palestine. It is distinctly shown how the blossom period of 1936 coincided with the aphis infestation.

A close survey of such an infestation was made in a grove in Ness Ziona, the following facts being revealed:

(1) Most of the trees attacked were well protected from the south so that the hot, dry desert winds blowing from that direction could not affect the aphis on them as readily as on other unprotected trees.

(2) As a rule, only the northern side of such trees was injured, while the southern side, facing the desert wind, was free from such infestation. Due to the retarding effects of the Khamsen winds, the aphid could continue their damage to a later date than would have been possible otherwise.

PRACTICAL SUGGESTIONS

A mild winter, when the daily maximum temperature during December and January is above the line of 18° C., may encourage the infestation of *Toxoptera aurantii*, which will become most abundant during the month of March.

If measures of control are to be taken it should be done during February, before the leaves have curled. A solution of nicotine sulphate 1 : 800–1 : 1000 proved to be satisfactory against this pest.

SUMMARY

1. The *Citrus* aphis reproduces parthenogenetically continuously throughout the year; sexual reproduction has not been observed.

2. The most favourable temperature for development and reproduction of the aphis is between 22 and 25° C. Between 18 and 22° C. the rate of reproduction and development is also sufficient to bring about heavy infestation.

3. The temperature below 18° C. is unfavourable for considerable increase of the population of this insect. At 7° C. reproduction is barely taking place.

4. At 30–32° C. the mortality of the nymphs is almost 100%. At 34–35° C. reproduction ceases, and at 36° C. adult insects die within a short period.

5. The annual fluctuations of the population of the insect based on field observations are described.

6. Food as a factor in the increase of the population of the aphis is discussed.

7. The effect of the annual fluctuations of the temperature in Palestine upon the population of the aphis is pointed out.

8. The economic status of the insect is discussed.

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STUDIES OF THE BIOLOGY OF THE DEATH-WATCH BEETLE, *XESTOBIUM RUFOVILLOSUM* DE G.

II. THE HABITS OF THE ADULT WITH SPECIAL REFERENCE TO THE FACTORS AFFECTING OVIPOSITION

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(With 6 Text-figures)

CONTENTS

Introduction

A GENERAL account of past work on the death-watch beetle, *Xestobium rufovillosum* De G., was given in the first paper (Fisher, 1937) of this series. The results of part of recent studies at Princes Risborough on the biology of the insect are discussed in the present contribution, which deals particularly with the adult and the effect of temperature and humidity on oviposition.

A serious handicap at the outset of the investigation was lack of insect material, for as Lefroy (1924) also found, beetles could not be obtained in sufficient numbers from timbers from buildings in which damage had been discovered. Moreover, the size of the timbers concerned and the problem of suitable storage space to allow of regular handling and examination rendered unsuitable such a doubtful source of supply of the insects. Abundant material was eventually obtained from decayed parts of willow trees along the banks of streams near Thame, Oxfordshire. Frequent visits were made to these trees, two of which were removed and transplanted at the Laboratory.

In addition to life-history studies carried out in the laboratory, inspections of buildings undertaken at the request of authorities in charge of repairs afforded valuable opportunities of studying the insect in the conditions under which it is often responsible for serious damage to structural timbers.

Pupal period and emergence

In the literature on *Xestobium rufovillosum* there is conflicting evidence as to the time of year at which emergence from the wood takes place and how this is related to the date of completion of the pupal period. For instance, in describing the life history of the insect in its natural habitat out of doors, Munro (1928) states that pupation occurs in spring but the beetles rarely come out of the timber until autumn or even the following spring. Lefroy (1924), forecasting the probable life cycle, indicates that pupae may be found during June and September, and that the beetles emerge in May and June of the following year. Gahan & Laing (1932), and Kimmins (1933–4) state that pupation occurs in late summer and early autumn but that the beetles do not emerge until spring. In view of these differences of opinion, observations have been made in the field, in buildings and on timber removed from buildings and subsequently stored under cover at the Laboratory. In discussing the results of this work it is convenient to deal separately with

the time of pupation and emergence of the insect under natural conditions out of doors, and under artificial conditions in buildings.

A. *Out of doors*

The earliest date at which pupae were found in the open in decayed parts of willow trees was 18 July (1935). During the remainder of this month, in August and occasionally in early September, pupae were found in the trees under observation over a series of years. The pupal period was determined by locating pupating larvae which were removed and kept out of doors and as Table I shows, lasted from 3 to 4 weeks, there being little difference between the sexes. According to Kimmins (1933-4) the period occupies about 3 weeks under "laboratory conditions".

Table I

Duration of pupal stage out of doors, 1935

Sex	Pupal period	Duration (days)	Av. temp. ° C.	Av. R.H. %
♂	20–23. vii. to 12. viii.	21–24	17·8	69
♂	20–23. vii. to 12. viii.	21–24	17·8	69
♀	5. viii. to 28. viii.	24	18·4	71
♀	7. viii. to 26. viii.	20	17·8	71
♀	5. viii. to 1. ix.	28	17·8	71

It follows from the above that from August onwards immature beetles are present in infested trees, but observations have shown that they remain inactive throughout the autumn and winter within their pupal cells or in honeycombed wood and normally do not emerge until April and May of the following year. The earliest dates at which emergence was noted were 25 April 1928, and 23 April 1935, but the majority of beetles emerge during May; a few do not appear until June. The following records of the average outside temperatures (in ° C.) at Princes Risborough during these months is of interest for comparison with those in buildings at the time of emergence of the beetles:

	March	April	May	June
1934	5·2	8·4	12·2	15·4
1935	6·7	7·7	10	16·1

The average temperature out of doors at the time of maximum emergence is, therefore, in the neighbourhood of 10–12° C., but emergence probably occurs only on days when the temperature is above the average for the period.

The progress of development of the reproductive organs, described by Fisher (1937), during this period of hibernation is worthy of note.

In the female the rate of development of the ovarioles is slow. For approximately 2 months after pupation no traces of oocytes are usually visible, whilst fat body is abundant. Subsequently, traces of two or three developing oocytes can be distinguished near the base of each ovariole and development proceeds so that by April, at the time of emergence, although not fully mature, two to three well-developed eggs are present in each ovariole; fat body is still present but not abundant.

In the male the progress of development is more striking by reason of the marked changes that occur in the shape and size of the testicular follicles. For a short time following completion of the pupal period the follicles are elongate and cylindrical. This stage of development was noted in beetles collected from trees only during August and early September. By the end of September or early October the follicles have lost their cylindrical appearance, become smaller and pear-shaped as their contents pass into the vasa deferentia and seminal vesicles, which as a result are distended by the seminal fluid. Throughout the remainder of the winter a further diminution in the size of the follicles takes place, until in beetles examined between December and April they appear as minute oval bodies visible only with difficulty. By this time fat body is practically absent and, at emergence, the males are sexually mature.

B. *In buildings*

It has not been possible to procure as full information upon the time of pupation and emergence of *Xestobium* from timber in buildings as from infested trees. Examination of timber shortly after removal from the roofs of churches has, however, shown that in such buildings, unheated or heated only intermittently throughout the winter, pupae may be found at approximately the same time of year as out of doors, viz. August and September. On the other hand, it has been found in the course of experiments in the laboratory that pupation may occur throughout the year, depending upon temperature, humidity and other conditions affecting the rate of development of the larvae. This aspect of the investigation is outside the scope of the present paper, but it is opportune to note the duration of the pupal stage (Table II), when the temperature and humidity at which the development of the earlier stages of the insect (egg and larva) were completed, are considerably higher than those normally prevailing in the open.

From Table II it is apparent that under such conditions the pupal period is about 1 week less than that out of doors, but these conditions are unlikely to occur in buildings unless artificially heated during the

normal time of pupation of the beetle in the late summer. A comparison of the average temperatures prevailing out of doors at this time and in buildings in which death-watch beetle activity was known to occur showed that little difference existed in the two situations. Although the extremes of temperature were greater out of doors, a marked prolongation of the pupal period did not result. It can, therefore, be concluded that the slight difference between the length of the pupal stage out of doors and in buildings has little effect upon the subsequent date of emergence of the adults and the duration of the life cycle as a whole.

Table II

Duration of pupal stage under experimental conditions

Sex	Pupal period	Duration (days)	Av. temp. ° C.	Av. R.H. %
♂	19. viii. to 4. ix.	16	23·9	89
♂	19. ix. to 6. x.	18	22·7	90
♀	15. ix. to 2. x.	18	22·7	90

The earliest date at which beetles appeared in a church kept under close observation in 1934 was 7 April. Other records of the occurrence of beetles in buildings have, however, been obtained from mid-March to early June, but the majority of the insects are to be found during the latter part of April and in the beginning of May. The average temperature recorded in the vicinity of infested timber in a building at the commencement of the emergence period was 10° C., which corresponds closely to that out of doors in May, the time of year at which maximum emergence takes place. Dissections of beetles taken from timber from infested buildings during the winter months showed that they also were sexually immature.

When examining timber stored outside for several months after removal from buildings, pupae were obtained on two occasions at times not in accordance with the general habit of the insect as described above. In the first instance, in April and May 1932, six partly coloured pupae, together with numbers of beetles, were cut from alder beams which had been removed from a cottage in June 1931. In April 1933, beetles were obtained but no pupae were then found. In the second case two pupae were taken at the end of January 1933 from deal flooring stored under cover out of doors after removal from a building in August 1931; beetles were again found at the same time as the pupae. A satisfactory explanation of these occurrences of pupae in the early spring has not been found, but is most probably linked up with the previous history of the timber upon which full information was not available, and its effect upon the

duration of the larval stage. In this connexion, mention has already been made of the effect of temperature and other conditions, as shown by experiment, on the duration of the larval stage of *Xestobium*, leading to pupation and emergence of the adults at abnormal times of the year. Moreover, it is to be noted that neither of the timbers concerned is commonly attacked by the death-watch beetle. The occurrence of *Xestobium* pupae in spring is evidently unusual and an exception to the normal life cycle of the insect out of doors or in churches.

These special instances apart, the results of the observations out of doors and in buildings confirm in general those of Gahan & Laing (1925), and of Kimmins (1933–4) that death-watch beetles may be expected to emerge and oviposit during the period mid-March to early June. The condition of the male and female reproductive organs at the time of emergence indicates that the males, which emerge before the females, are sexually mature and capable of proceeding at once to pairing. The females, although not so advanced in development, are rapidly approaching maturity and, as shown later, are ready for copulation. The adults of both sexes do not bore after emergence nor do they feed. Under natural conditions out of doors the sexes occur in approximately equal numbers. For instance, dissection of 167 beetles collected from willow trees in March 1934 showed eighty-six to be males and eighty-one females.

Although emergence is restricted to a definite time of year, normally there is no period at which the insects appear in large numbers. Observations and experiments have shown that the beetles usually emerge a few at a time, and unless looked for carefully, their presence either on the surface of infested trees or in a building can easily be overlooked. On the other hand, in cases of severe infestation when timbers are in a condition especially suitable for attack, a big emergence may take place over a period of several days depending upon temperature conditions. It has been suggested by Lefroy (1924) that true emergence may not always take place, and that in severely damaged timber in a honeycombed condition egg-laying occurs without the parents leaving the timber in which they completed their development. Whilst no definite proof of this has been obtained, support is given to the suggestion by the finding of adults during the normal time of egg-laying within tunnels in oak structural timbers and by the extreme difficulty with which beetles have been collected without cutting up and submitting the timber to a most detailed examination.

HABITS OF ADULTS

Activity

The beetles are normally inactive and out of doors are usually to be found on the underside of loose pieces of bark, in old tunnels or other sheltered places in the vicinity of attacked wood. On sunny, warm days, they have been seen wandering over the surface of attacked trees, tapping frequently; the time of maximum activity is in the late afternoon from 4 to 6 p.m. Kimmins (1933–4) suggests that they are most active during the night, but this is not confirmed by our observations. On the contrary, it appears that activity is governed largely by temperature and by sunlight. For instance, beetles kept in the laboratory were active and moved about slowly at a temperature of 14° C., but their activity greatly increased when the temperature rose to 17–20° C., tapping, pairing and egg-laying being observed.

Tapping

The characteristic tapping produced by the beetle has been the subject of discussion in earlier literature (Allen, 1695; Derham, 1701; Westwood, 1839–40; Altson, 1922; Kimmins, 1933–4) and does not call for special comment. It is sufficient to record that our observations confirm those of the later workers and that the sound is produced by the insect rapping its frons seven or eight times in quick succession on the surface of the wood and is probably a sex call. Accordingly, it is heard only during the months of emergence, prior to and during oviposition. Tapping takes place when the insects are most active and therefore depends upon the same conditions as those which govern their activity. Although both sexes tap, the male does so more frequently; dissection of a batch of insects noted tapping revealed nineteen males and only two females. Tapping may continue after pairing.

Flight

It is questionable whether the beetles ever fly intentionally. They are capable of flight or at least of using their wings to break a fall or to regain their feet when they have fallen on their backs, but there is no evidence to show that their powers of flight are of importance as a means of spread of infestation, e.g. from building to building. On the other hand, the frequency with which beetles have been found on the floors of churches, of which the roofing timbers were known to be attacked, suggests that they might have originated from the roof, and in the absence of any

visible evidence of flight dropped to the floor below, there to congregate in cracks and crevices at the base of pews, rails, etc. They are capable of crawling considerable distances, and in this way may spread infestation within a building.

Pairing

Although pairing was observed at all times of day and evening, both out of doors and in the laboratory, it was recorded most frequently in the late afternoon and was dependent on conditions favouring the activity of the beetles. Copulation was seen when both insects were on the surface of the wood, but it is probable that it can also take place within the tunnels of extensively honeycombed timber. The male mounts upon the back of the female, and when coition has been established gradually falls over backwards and finally rests on its back, with legs and antennae drawn closely into the sides of the body. The female is capable of supporting the male in this position and was seen on occasion to crawl over the surface of the wood with the male thus attached. At laboratory temperature (18–20° C.) the duration of copulation varied from $\frac{1}{2}$ to $1\frac{1}{4}$ hr.

Dissections of females after pairing have shown that the uterus is at first much distended and globular in shape. The contents are white, opaque and of a glutinous consistency. Gradually, however, these disperse and the uterus, passing through an intermediate stage when it becomes pear-shaped, being partly distended and opaque anteriorly but elongate and translucent posteriorly, assumes its normal appearance after 4–6 days, under out-of-door conditions. The presence of a small yellow-brown body in the cavity of the bursa copulatrix has frequently been noted during dissection of females at different stages of development. This substance is absent before and immediately after pairing but is invariably present in fertilized females in which the uterus has regained, or almost regained, its normal appearance, irrespective of whether or not egg-laying has taken place. The origin or significance of this substance has not been determined.

Oviposition

Methods of study

In studying oviposition and the duration of the egg stage, an endeavour has been made to determine the effect of different relative humidities and temperatures with the object of assessing the importance of these factors in relation to the duration of the life cycle of the insect in buildings and out of doors. Numbers of beetles, segregated into individual pairs

whenever possible, were confined in a series of vessels kept at different temperatures, and in which the relative humidity of the atmosphere was controlled by mixtures of sulphuric acid and water (Wilson, 1921). In addition, beetles were placed in other vessels out of doors and meteorological records kept. The material offered to the insects for oviposition was oak sapwood and willow, previously decayed by *Phellinus cryptarum* Karst and *Ganoderma applanatum* (Pers.) Pat., respectively, each fungus producing a white rot. Sound oak sapwood and willow were also used as controls. The choice of decayed timber was the outcome of experiments which showed that wood in such a condition was specially suitable for the development of the larvae of *Xestobium*. The samples were placed in the vessels to condition to an equilibrium moisture content in accordance with the relative humidity of the atmosphere in each.

In view of the habit of the insect of over-wintering in the adult stage, and in order to be certain that the beetles used had not paired before collection, it was usually necessary to remove them from infested timber or trees during the winter months and keep them under close observation in the different environmental conditions concerned. The absence of external sex characters was a serious handicap in this work.

Mode and location of egg-laying

Oviposition was never observed, nor have eggs been found in timber under natural conditions in trees and in buildings. On the few occasions when egg-laying was observed in the laboratory the female beetle moved slowly over the surface of the wood exploring all pits and crevices, first with her antennae and then, turning round, with the palps at the tip of the ovipositor which was protruding slightly beyond the everted last abdominal tergite. On locating a suitable crevice the ovipositor was extruded and inserted therein: about 5–7 sec. elapsed before contraction of the abdomen and sudden progressive dilatation of the ovipositor showed that an egg was being deposited. Several such crevices were carefully marked and subsequent examination showed an egg in each. In one case under observation, a female seen to be ovipositing at 11.15 a.m., when the laboratory temperature was 20° C., continued egg-laying at intervals throughout the day.

In order to determine whether oviposition is more frequent during day or night, counts were made of the numbers of eggs laid by individual beetles throughout their life, between 10 a.m. and 10 p.m. and between 10 p.m. and 10 a.m., in the laboratory and out of doors, during June and July 1936. The results showed that although occasionally eggs were laid

between 10 p.m. and 10 a.m. the following day, by far the greater number were laid during the day and early evening. In view of the observed activity of beetles in the late afternoon it is probable, therefore, that egg-laying takes place most frequently at that time.

In experiments in the laboratory, eggs were inserted singly, in pairs and sometimes in small groups, in cracks or crevices, on roughened surfaces, among broken fibres of wood, preferably on the transverse surface. They were also noted within the vessels of oak, and in all situations were inserted as deeply as possible so that it was frequently difficult to detect their presence. Eggs laid under loose chips were invisible unless these were raised or pulled to one side. Instances were also recorded of the presence of eggs in the frass of old tunnels. It was not uncommon to find groups of eggs varying in number from five to six up to as many as 150 on the exposed surfaces of samples, more frequently between adjacent specimens, or on the under surface between the sample and floor of the vessel. When in groups, each egg was attached to, or at least in contact with, its neighbour and adhered to the substratum probably by a cement on the chorion which set soon after the egg was laid.

From these observations it seems probable that, in timber in buildings, beetles oviposit in a great variety of situations and conceal their eggs by whatever means are offered, according to the condition of the wood, by inserting them in cracks, crevices and open joints at the junction of individual timbers, or hiding them among old larval frass. There is no evidence to suggest that the choice of site for oviposition can be correlated with the suitability of the surrounding timber for the young larvae to commence boring. On the contrary, beetles offered a choice of decayed and sound oak sapwood, each provided with cracks and crevices, did not show a preference for ovipositing in the decayed specimens. The ability of the larvae, on hatching, to crawl over the surface of the timber, presumably in search of a suitable place to commence boring, compensates for any handicap resulting from oviposition having taken place in a situation unsuitable for their future development.

Records of egg-laying and duration of egg stage

In the experimental work on oviposition and the duration of the egg stage, accurate records were frequently difficult to obtain on account of the position of the eggs. This source of experimental error, must, therefore, be taken into account in considering the results obtained. It is convenient to deal separately with the observations and experiments

out of doors and under controlled conditions of temperature and humidity.

A. *Out of doors*

Records of egg-laying were obtained by keeping beetles, including segregated pairs, under observation in muslin-covered glass dishes. In some instances desiccators were used in which the relative humidity was maintained at 86 %. A summary of the results is given in Table III.

Table III

Egg-laying records out of doors

Exp. started	No. of beetles ♂	No. of beetles ♀	Date paired	Date 1st eggs laid	Av. duration of egg-laying (days)	Av. eggs per ♀
30. i. 33*	5	4	–	29. iv. 33	46	44
31. i. 33*	2	5	–	27. iv. 33	65	60
6. ii. 33*	3	4		29. iv. 33	63	50
4. iv. 34	5	2		15. v. 34	18	77
8. v. 34	1	1	8. v. 34	17. v. 34	16	75
8. v. 34	1	1	8. v. 34	15. v. 34	12	54
8. v. 34	1	1	8. v. 34	21. v. 34	16	59
23. iv. 35†	1	1	23. iv. 35	28–30. v. 35	24	140
25. iv. 35†	1	1	25. iv. 35	28–29. v. 35	25	39
25. iv. 35†	1	1	25. iv. 35	31. v. 35	36	108

* At 86 % relative humidity.
† Found pairing on surface of tree but may have paired previously.

The interval of 8–13 days between pairing and egg-laying, recorded in Table III, agrees comparatively closely with that of 5–11 days given by Kimmins (1933–4), who does not, however, state whether his figures refer to insects kept out of doors. The observations in general suggest that this period is governed by a number of factors amongst which the most important are the state of development of the ovaries at the time of pairing and temperature conditions during subsequent weeks. For instance, other records show as many as 36 days to elapse between pairing and egg-laying, and in this connexion it is significant that in two experiments started in 1934 at an interval of 1 month, egg-laying commenced in both at approximately the same time (May) and continued for 2–3 weeks, during which the number of eggs laid per female varied from fifty-four to seventy-seven. It is of interest to compare with these results the egg-laying records obtained in 1933 from the three experiments in which the relative humidity was kept at 86 %, rather higher than that (approximately 70 %) of the open air at this time of year. Egg-laying began about the same time in each, but earlier than in 1934 and continued over a much longer period, 6–9 weeks, but the average number of eggs

per female was only forty to sixty. Meteorological data were not available at Princes Risborough in 1933, but it is significant that, according to the monthly returns of the Meteorological Office, temperatures in general from March to May of that year were considerably higher than those for the corresponding period in 1934. The date of commencement and the duration of egg-laying are, therefore, probably correlated with temperature conditions. Further evidence in support of this has been obtained from experiments described later.

It is also evident from Table III that there is considerable variation in the number of eggs laid per female. For instance, in the records of oviposition of individual pairs of beetles, the number of eggs per female varied from as few as thirty-nine to as many as 201. The usual number of eggs per female was forty to sixty. It should be explained that, as a result of a difference in fecundity of individual beetles, as affected, for instance, by a variation in the number of ovarioles per ovary (Fisher, 1937), the potential egg-laying capacity of different females will vary considerably. Moreover, as is shown later, conditions of temperature and humidity also play an important part in this respect.

When it was possible to determine accurately the length of life of individual beetles, observations showed that the females may live up to 10 weeks after pairing; the males are shorter lived, surviving 8–9 weeks after pairing. In no case under out-of-door conditions were beetles of this generation found alive after the beginning of July. It will be recalled that pupation is completed in July and August, 7–8 months before the beetles emerge from the wood for pairing and egg-laying. The total length of adult life of the death-watch beetle is, therefore, from 10 to 11 months, during only three of which, April-June, egg-laying takes place.

Incubation period of the egg. The duration of the egg stage was determined by examination of the samples from which egg-laying records were obtained. The mean duration of the stage ($\bar{x}$), given in Table IV was calculated as

$$\bar{x} = \frac{\Sigma n_1 r_1}{n},$$

where n_1 is the number of eggs hatching after a recorded period, r_1 the duration of this period in days, and n the total number of eggs hatching.

A comparison of the records in Table IV, in which the egg period varies from 3 to 5 weeks, with the mean temperature at Princes Risborough from May to July in 1934 and 1935, suggests that this variation is due to the differences in temperature prevailing throughout the egg stage

Table IV

Duration of egg stage out of doors

Date of oviposition	Eggs	Mean duration of stage (days)	Av. temp. ° C.
1934: 15. v. to 1. vi.	297	35·7	14·5
1935: 28. v. to 31. v.	136	35·4	15·6
15. vi. to 21. vi.	114	25·2	18·3
23. vi. to 5. vii.	8	21·9	18·7

according to the date of oviposition. For instance, the average temperature out of doors during the development of the 136 eggs laid in the end of May was 14·5° C. as compared with 18·3° C. during the incubation period of the 114 eggs laid in the end of June and beginning of July. The effect of constant temperature and humidity on the duration of the egg stage is discussed later, but it is evident from the above records that out of doors this period is normally about 5 weeks, since the majority of the eggs are laid before the end of May.

Viability of eggs. The viability of the eggs as determined by the percentage hatching after allowance has been made for those accidentally destroyed or lost is given in Table V.

Table V

Viability of eggs out of doors

Year	No. of eggs	% hatched
1934	·315	97·8
1935	339	93·3

Average R.H. during egg period – 70% (approx.). Range of average daily temperature during egg period = 14–21° C.

The average survival of *Xestobium* eggs out of doors is therefore about 95%.

B. *Under controlled conditions of temperature and humidity.*

In order to obtain further information upon the effect of temperature and humidity on oviposition, a series of experiments was carried out at relative humidities between 23 and 95% and at temperatures of 15, 20, 25 and 30° C. Some difficulty was experienced in maintaining a constant temperature of 15° C., fluctuations of ± 3–4° C. occurring, but the mean temperature was approximately correct. The majority of the experiments were started at the same time during April and May, the normal period of emergence and egg-laying, and are therefore supplementary to those described above under out-of-door conditions. As far as could be determined by size, in the absence of secondary sex characters, four pairs

of beetles were used in each experiment; the sex of all individuals was ascertained by dissection at the conclusion of the work. In discussing the results which are summarized in the following tables, it should be noted that the records were obtained from observations on groups of insects, the individuals among which varied in egg-laying capacity, duration of life, etc. Such variations in themselves may mask the effect, if any, which conditions of temperature and humidity exert, but it is believed that sufficient data are available to enable general conclusions to be drawn.

Table VI

Effect of temperature and humidity on interval between emergence and oviposition

Exp. started	R.H. %	Interval (days) between start of exp. and date of first egg-laying: 15° C.	20° C.	25° C.	30° C.
4–5. iv. 34	23	18–21	7–8	7	9
4–5. iv. 34	41	18–21	9	6–7	6
4–5. iv. 34	53	23–26	7–8	8	6
4–5. iv. 34	75	18–21	9–10	8	7
25. i. 33*	86	—	—	12–13	—
1. ii. 33*	86	—	—	13	—
6. ii. 33*	86	—	15	—	—
21. viii. 33*	86	—	—	80–90	—
29. ix. 33*	86	—	126	—	—
4–6. iv. 34	95	14	10	8	6

* Beetles used removed from wood prior to or during hibernation.

It is evident that, humidity apart, temperature is an important factor in determining the date at which egg-laying commences. Thus, the records show in general that during the normal egg-laying period (May) an increase in temperature results in earlier oviposition. The effect of a difference in temperature of 5° C. at 15 and 20° C. is most marked, but above 20° C. is less pronounced. It is also apparent that a temperature considerably above that which normally prevails in the open can result in egg-laying taking place during the winter months. Under such circumstances the date of first egg-laying is also governed by the state of development of the beetle at the time of exposure to the increase in temperature. For instance, when beetles taken from pupal chambers in trees in August and September, i.e. about 1 month after completion of the pupal period, and therefore before hibernation, were exposed to temperatures of 25 or 20° C. and a relative humidity of 86 %, the earliest dates of egg-laying were during November and the following January respectively.

Relative humidity does not appear to be an important factor in determining the time of oviposition.

Duration of egg-laying and number of eggs laid per female. The effect of temperature and humidity on the duration of egg-laying and on the number of eggs per female is shown in Table VII.

Table VII

R.H. %	Duration of egg-laying (days) 15° C.	20° C.	25° C.	30° C.	Av. no. of eggs per ♀ 15° C.	20° C.	25° C.	30° C.
23	23–26	17–18	8	9	30	60	20	7
41	23–26	15	15–16	10	34	54	23	24
53	18–21	18–19	9	15	31	45	45	24
75	34–37	24–25	21	14	52	37	45	45
95	31	41	17	24	61	79	115	50

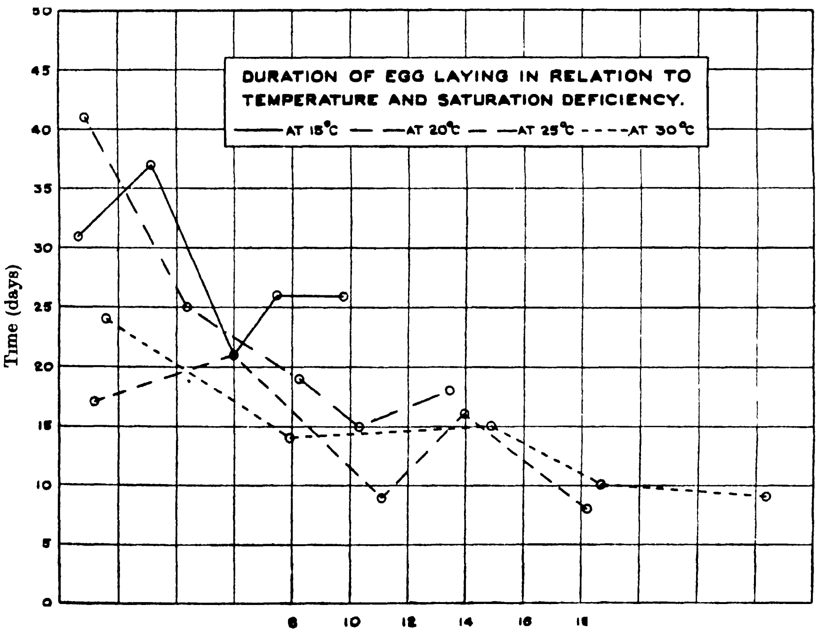


Fig. 1

These records show that between 15 and 30° C. there is a tendency for the egg-laying period to be prolonged as the relative humidity rises, accompanied, at the higher humidities, by an increase in the number of eggs per female. An increase in temperature tends to shorten the egg-

laying period, and up to 20° C. to increase the number of eggs per female. There are indications that at a higher temperature the number of eggs per female decreases when the humidity is low. If, however, the humidity increases, the oviposition records also increase up to 20–25° C., thereafter falling off. These observations suggest therefore that temperature and humidity together play an important part in governing oviposition and

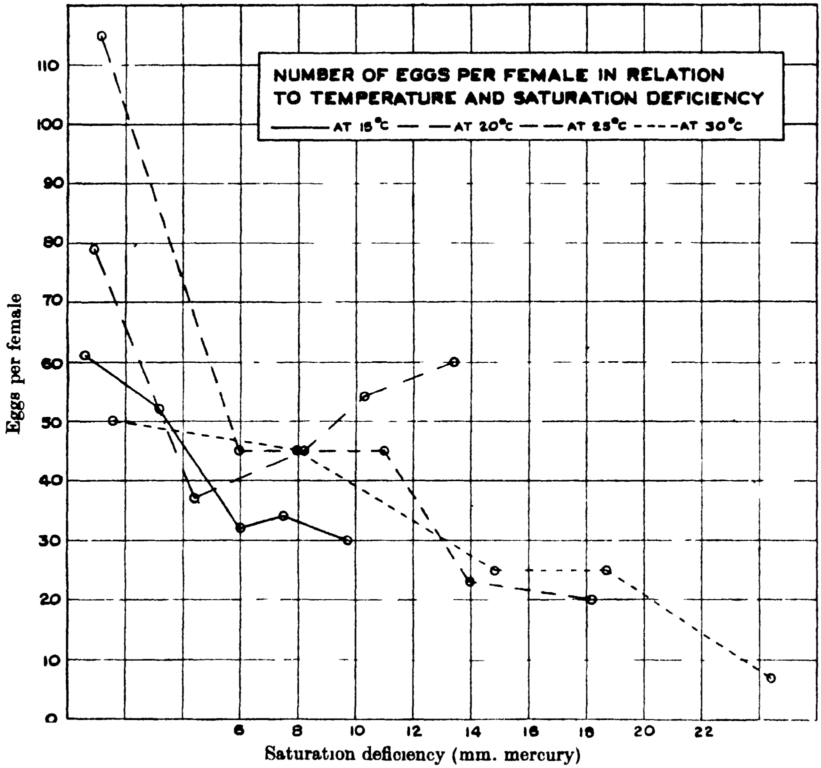


Fig. 2

its duration, the lower temperatures accompanied by high humidities being more favourable. As Buxton (1931) has suggested, such an effect can best be shown by comparing the results with the saturation deficiencies corresponding to the different temperatures and humidities used. If this be done (Figs. 1 and 2) it becomes evident that, in general, dry conditions curtail the duration of egg-laying and decrease the number of eggs per female. Furthermore, the effect of various temperatures, as apparent in

Table VII, is entirely explained by the different saturation deficiencies which they cause.

Duration of life of adults after emergence. Records were kept of the length of life of the beetles used in the oviposition experiments, and the results, summarized in Table VIII, refer to this period as calculated from the commencement of these experiments in April at the normal time of emergence and the start of egg-laying.

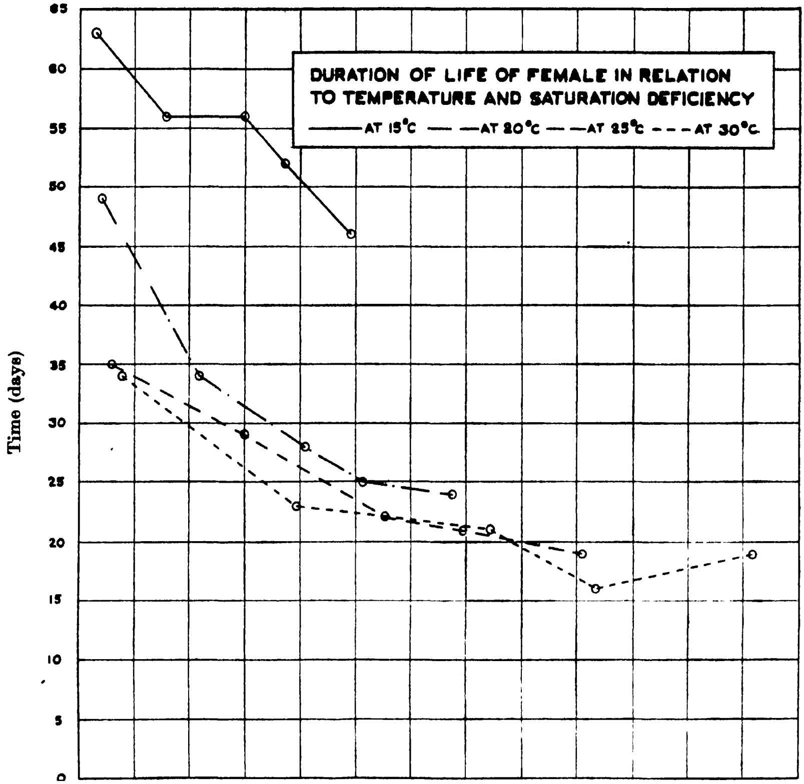
Table VIII

Average duration of life of adults (days)

R.H.	15° C.		20° C.		25° C.		30° C.	
%	♂	♀	♂	♀	♂	♀	♂	♀
23	36	46	19	24	17	19	14	19
41	44	52	21	25	14	21	14	16
53	42	56	22	28	20	22	16	21
75	52	56	24	34	19	29	15	23
95	36	63	27	49	22	35	15	34

These figures show that the length of adult life again varies according to conditions of temperature and humidity. Thus, at any one humidity, an increase in temperature shortens the period, but at a given temperature a rise in humidity has the opposite effect, tending to prolong adult life. At the lower temperatures, 15–20° C., approximating to those out of doors, the duration of life of the female varied, according to conditions of humidity, from 7 to 9 weeks approximately; under similar conditions the males lived from 5 to 7 weeks. At a temperature of 30° C., on the other hand, the duration of life of the female was only 3 to 5 weeks, and that of the male about 2 weeks. These conclusions are confirmed by examination of the curves (Fig. 3) showing the relation between the duration of life of the female and the saturation deficiencies corresponding to the temperatures and relative humidities cited in the above table. As the saturation deficiency decreases adult life is prolonged. Furthermore, the effect of temperature is not entirely included in the changes in saturation deficiencies as shown in these curves, since an increase in temperature from 15 to 20° C. shortens the length of life of the female but a rise from 20 to 30° C. has little further effect.

These observations support the conclusions reached from the out-of-door experiments, and show that the active life of the adults is confined to a comparatively short period in late spring and early summer. Thereafter, they die off, none surviving later than early July.



Saturation deficiency (mm. mercury)

Fig. 3

Incubation period of the egg. The effect of temperature and humidity on the duration of the egg stage is shown in Table IX and represented graphically in Fig. 4.

Table IX

Duration of egg stage (days)

Temp. °C.	R.H. % 23	41	53	75	86	95
15	No hatching *(91)**	49·7 *(132)*	41·3 *(78)*	45·6 *(189)*	—	44·9 *(80)*
20	No hatching *(119)*	23·6 *(123)*	21·3 *(82)*	20·01 *(73)*	20·7 *(83)*	21·1 *(399)*
25	No hatching *(118)*	15·3 *(41)*	15·7 *(18)*	14·2 *(133)*	15·7 *(180)*	12·5 *(96)*
30	No hatching *(29)*	*(118)*	*(116)*	*(178)*	*(22)*	*(248)*

* Figures in italics refer to numbers of eggs.

At each of the temperatures stated, changes in relative humidity above 41 % have little or no effect on the rate of development of the egg. At 23 % on the other hand, at all the temperatures quoted, hatching did not take place. The lowest humidity at which egg development can proceed was not determined, but lies between 23 and 41 % within the limits of the above temperatures. If, however, the duration of the egg stage is plotted (Fig. 4) against the saturation deficiencies corresponding

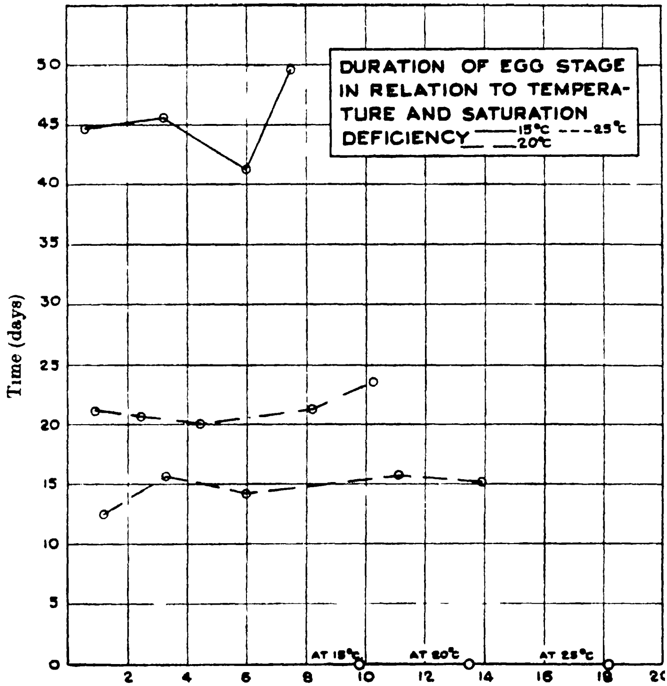


Fig. 4

to the different relative humidities at each temperature, it is evident that the degree of dryness which the eggs can tolerate varies with the temperature. Within the range of effective temperatures, a rise brings about an increase in the degree of dryness that can be tolerated by the eggs without affecting the duration of the stage, thus:

Temp. ° C.	Max. saturation deficiencies for completion of egg state (mm. of mercury)
15	Between 7·5 and 9·5
20	„ 10·3 „ 13·5
25	„ 13·98 „ 18·2

Furthermore, it is evident (Fig. 4) that although dryness has only a limiting effect on the duration of the egg stage, temperature, as the results of observations out of doors suggest, has a progressive effect. Whilst the minimum fatal temperature has not been determined, it lies

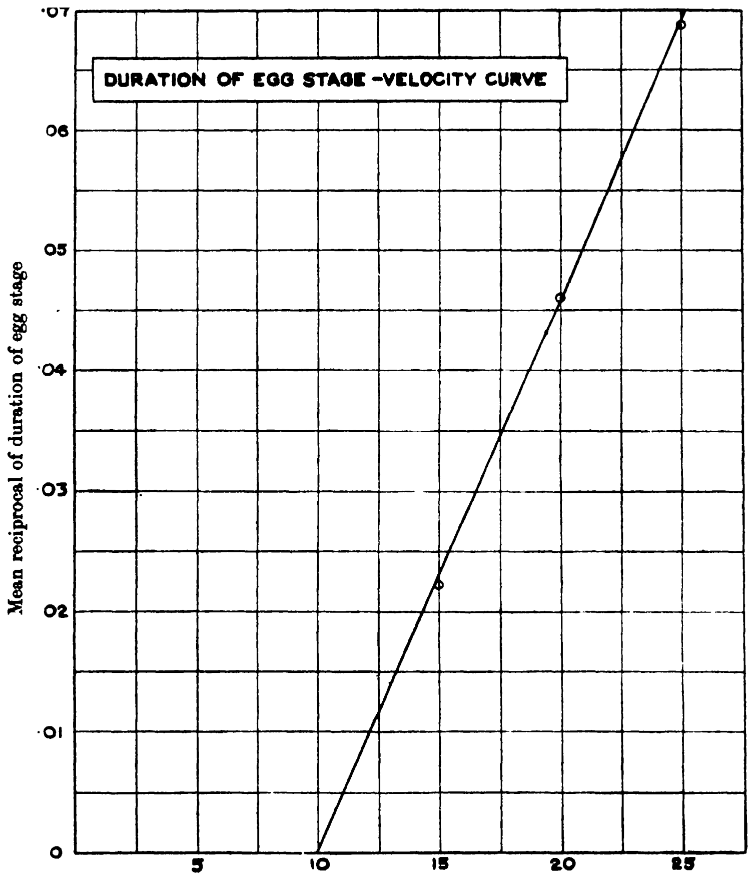


Fig. 5

between 25° C., at which the egg period lasted 2 weeks, and 30° C. at which hatching did not take place. Moreover, it seems probable that 25° C. is approaching the optimum temperature for the duration of the egg stage, since under such conditions the egg hatches in a shorter time and can tolerate greater dryness than at lower temperatures. The

separation of the curves at 15 and 20° C., and at 20 and 25° C. supports this suggestion. At the other end of the temperature range under investigation, the mean duration of the stage was approximately 7 weeks at 15° C. A few eggs kept at 10° C. had not hatched after 8 weeks, but when subsequently exposed to a temperature of 20° C. completed their development.

These observations suggest that the threshold of development of *Xestobium* eggs lies between 10 and 15° C. The theoretical threshold as shown in the velocity curve (Fig. 5) obtained by plotting the mean reciprocals of the duration of the egg stage against temperature is in the neighbourhood of 10° C. It is significant that this temperature approximates to that which normally prevails out of doors at the beginning of the emergence period of the beetles at the end of April and during May.

Viability of eggs. The records of viability obtained in the oviposition experiments under controlled conditions in the laboratory are summarized in Table X.

Table X

Viability of eggs

R.H. %	15° C.	20° C.	25° C.	30° C.
23	0	0	0	0
41	85·2	61·3	73·2	0
53	91·3	95·9	87·4	0
75	97·0	92·8	76·8	0
95	86·1	96·4	90·5	0

Very low humidities are unsuitable for the development of the egg; high humidities, on the other hand, are favourable but small changes in humidity at the upper end of the scale do not greatly affect the survival rate. The above figures also show the effect of temperature. Thus, at 25° C., the percentage survival is in general less than at lower temperatures, whilst at 30° C. the lethal temperature, irrespective of humidity conditions, has been passed. If the percentage survival of the eggs is plotted against saturation deficiency (Fig. 6), it is evident that very dry conditions are again unfavourable. Furthermore, at any of the given temperatures, an increase in saturation deficiency has little effect upon viability until a limiting value is reached when viability falls rapidly. Temperature is again seen to have an effect, not only in that the lower temperatures are slightly more favourable for hatching than the higher, but also as a study of the egg period has already shown (Table IX; Fig. 4) that the higher the temperature the greater the degree of dryness tolerated by the egg.

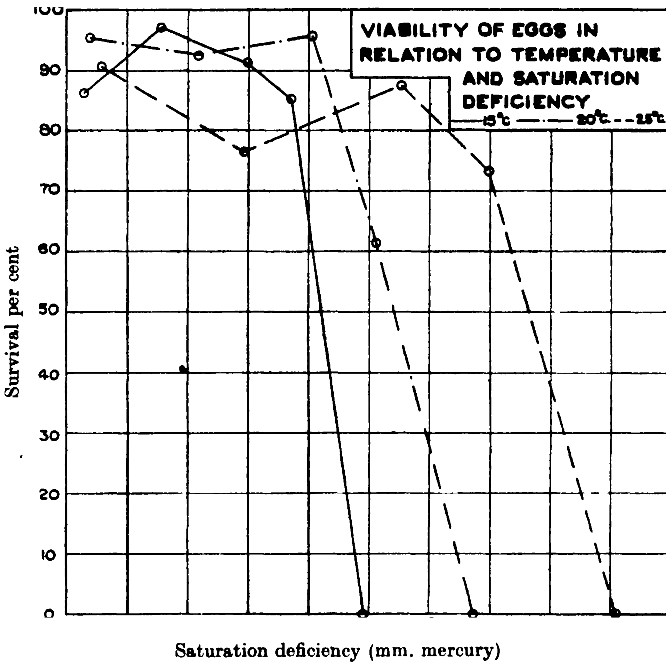


Fig. 6

Discussion

The effect of the different conditions of temperature, humidity and their related saturation deficiencies, on oviposition and life of the adult are summarized in Table XI.

So far as the rate of increase of *Xestobium* is dependent upon conditions favourable for oviposition and the hatching of the egg, the ultimate effect of temperature and humidity can best be determined by assuming that the most favourable conditions are those which allow the maximum number of viable eggs to be laid and hatched in the shortest time. This effect can conveniently be expressed as an "index of suitability" (I) for oviposition and hatching, and can be calculated in terms of the number of eggs laid (N), viability (V), duration of egg laying (L) and the egg period (T) for each combination of temperature and humidity according to the formula

$$I = \frac{NV}{L+T}.$$

Table XI

	Temperature 15–30° C.	R.H. 23–95 %	Sat. def. (dryness) (0·64–24·4 mm. mercury)
Duration of egg-laying (L)	Curtailed by increase in temperature, especially at low R.H. %	Prolonged by rise in humidity	Prolonged by decrease in dryness
Number of eggs per female (N)	Increased by rise in temperature up to 20° C. (approx.); thereafter decreased	Slight increase at high humidities	Increased by decrease in dryness. No temperature effect other than that represented by saturation deficiency
Duration of life of adults	Curtailed by rise in temperature	Prolonged by rise in humidity	Prolonged by decrease in dryness; additional temperature effect pronounced between 15 and 20° C.
Viability of eggs (V)	Decreased at high temperatures	Increased by rise in humidity	Little effect at low saturation deficiency; high saturation deficiency unfavourable, depending on temperature
Duration of egg stage (T)	Curtailed by rise in temperature. No hatching at 30° C.	Not affected by humidities above 41%. No hatching at 23%	Limiting effect only, varying with temperature

The most favourable conditions are those which yield the highest index. It is obvious that the numerical value of these indices would be altered if the whole life cycle were taken into consideration. They do however provide a means of comparing the effect of the different conditions on the rate of increase of the insect, during the stages under discussion.

Table XII

Indices of suitability for oviposition, etc., in relation to temperature and relative humidity

Temp. ° C.	R.H. % 23	41	53	75	95
15	0	0·38	0·47	0·61	0·69
20	0	0·85	1·04	0·76	1·27
25	0	0·54	1·57	0·99	3·6
30	0	0	0	0	0

The figures in Table XII show, in general, that a rise in temperature at relative humidities of 41 % and above, to an undetermined maximum between 25 and 30° C., favours an increase in the rate of reproduction of the insect. In addition, there is a general indication that at any one temperature an increase in humidity is also favourable. The optimum conditions appear to be a temperature near 25° C. combined with a high humidity.

By grouping the indices of suitability according to the saturation deficiencies at which they were determined (Table XIII), it is apparent that dryness is unfavourable and that the optimum conditions for egg-laying and hatching must include a low saturation deficiency.

Table XIII

Indices of suitability for oviposition, etc., in relation to saturation deficiency

Temp. °C.	Saturation deficiency (mm. of mercury) 0–4	4–8	8–12	12–16	16 and above
15	0·65	0·43	0	—	—
20	1·27	0·76	0·95	0	—
25	3·60	0·99	1·57	0·54	0
30	0	0	0	0	—

Whilst these figures show that within any one range of saturation deficiency, an increase in temperature to a maximum between 25 and 30° C. is favourable, they also demonstrate the increasing tolerance of dryness associated with a rise in temperature, to which attention has already been directed. Such a conclusion is in agreement with Buxton's (1931) suggestion that the body temperature is a factor controlling the activity or development of an insect. On this supposition the two variables, air temperature and air dryness, may be combined into one effect on body temperature. The tolerance of an increasingly dry atmosphere, as the temperature rises, is explained by the fact that evaporation causes lowering of the body temperature. Thus, increasing the dryness of warm air would assist the insect to accommodate its body temperature to unfavourable air temperatures.

The general conclusions arising out of this study of the factors affecting the oviposition of the death-watch beetle are of importance in relation to its occurrence and spread in buildings. For instance, warm dry conditions are less favourable for the spread of infestation during egg-laying than warm moist conditions which may be localized in structural timbers as the result of a leaking roof or imperfectly water-tight gutters. In this connexion, central heating may produce conditions suitable for egg-laying and hatching, in roofing or other timbers, by causing condensation of moisture unless adequate ventilation is provided. Furthermore, these findings offer a possible explanation of the slow progress or even cessation of attack which is often a feature of infestation of timber in buildings by the death-watch beetle. It is possible that high temperatures, associated with fluctuations in humidity, may occur in confined spaces in the roof of buildings and coincide with the period of

emergence and egg-laying, resulting in low egg production and a high egg mortality. Moreover, it is interesting to recall that of the Anobiidae in this country, *Xestobium* emerges in its natural habitat out of doors earlier than most other members of this family and is therefore apparently a species which for egg-laying does not like the higher temperatures prevailing later in the year. Consequently, although temperatures above those occurring in the open may favour oviposition, it is conceivable that as a result the rate of increase of the beetle may ultimately be decreased. In this connexion, Park (1935) has shown that the fecundity of *Tribolium confusum* is markedly affected by exposing the larval stages to a temperature of 39° C. for 5 hr.; the fertility of the beetle is however not affected. In the course of investigations now in progress, on the duration of the life cycle of the death-watch beetle, some evidence has been obtained which suggests that rearing this insect at temperatures above those occurring in its natural habitat may have a similar effect on its rate of reproduction in later generations. This also may be an explanation of the gradual cessation of attack in buildings.

SUMMARY

1. This paper, the second in a short series on biological studies of the death-watch beetle, *Xestobium rufovillosum* De G., is concerned with the life of the adult, egg-laying and hatching.

2. Times of pupation and emergence in the natural habitat of the insect in decayed parts of willow, and in timbers in buildings are discussed and compared.

3. The rate of development of the reproductive organs after pupation and until egg-laying starts, is described.

4. The habits of the adults, activity, tapping, flight and mode of pairing are commented upon.

5. The major part of the paper deals with oviposition which has been studied out of doors and under controlled temperature and humidity in the laboratory.

6. Out of doors forty to eighty eggs per female are laid during May and June over a period of 6–9 weeks. The egg stage lasts from 3 to 5 weeks, according to temperature conditions.

7. In the laboratory, oviposition and the hatching of the egg were studied at 15, 20, 25 and 30° C. and relative humidities of 23, 41, 53, 75, 86 and 95 %. The results are discussed in relation to the effect of temperature, relative humidity and saturation deficiency.

8. "Indices of suitability" for various combinations of temperature

and humidity in relation to egg-laying and hatching are calculated from the experimental data.

9. It is shown that an increase in temperature to a maximum between 25 and 30° C. is favourable but that the optimum conditions must include a low saturation deficiency. The data demonstrate an increasing tolerance, by the insect, of dryness associated with a rise in temperature.

10. The application of these findings to the occurrence and spread of *Xestobium* in buildings is briefly discussed.

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ON THE BIONOMICS AND STRUCTURE OF SOME DIPTEROUS LARVAE INFESTING CEREALS AND GRASSES

III. *GEOMYZA* (*BALIOPTERA*) *TRIPUNCTATA* FALL.

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(With 10 Text-figures)

CONTENTS

I. Introduction

The imago of *Geomyza tripunctata* Fall. closely resembles that of *G. combinata* L., and without microscopical preparations it is very difficult to tell the two species apart. According to Balachowsky & Mesnil (1935), however, this can easily be done by a microscopical examination of the male claspers.

Czerny in Linder's *Die Fliegen der Palæarktischen Region* (1928) describes the adults of *G. tripunctata* and *G. combinata*, but it is probable

that Czerny's *G. tripunctata* includes both *G. tripunctata* and *G. combinata*. The author has examined a number of geomyzids in the Cambridge museum which had been classified according to Czerny; the specimens labelled *G. tripunctata* were all that species, and the specimens labelled *G. combinata* were all *G. Balachowskyi* Mesnil. No *G. combinata* were found. Of the specimens bred during the course of this investigation by far the greatest number have been *G. tripunctata* (at first these were thought to include both *G. tripunctata* and *G. combinata*). A few *G. Balachowskyi* have been found but no *G. combinata*.

Since much confusion has existed in the identification of these species, it is possible that the description of the third-instar larva given by Frew (1923) as that of *G. combinata* is really *G. tripunctata*; he records the larva from wheat, barley, *Agropyrum repens*, *Festuca elatior*, *Lolium perenne*, *Holcus lanatus* and *Agrostis alba*. Miles (1913) has also recorded the damage done to *Lolium perenne* by the larva of *Geomyza tripunctata*. Kreiter (1928) has given a short account of the biology of this species and states that there is one generation per annum, the adults appearing during the first days in July. He figures the third-instar larva with only eight projections to the anterior spiracle. Balachowsky & Mesnil (1935) have lately given a short description of the larva of *G. tripunctata*, stating that it often attacks grasses of the genus *Lolium* to a very serious extent. In France there are two generations per annum, the first adults appearing in April.

II. Bionomics

(a) *Field observations*

In the field, adults of *Geomyza tripunctata* may be caught as early as the second week in March and as late as the last week in November. They can be swept from almost all grasses, in mid-field, near hedgerows or along roadsides. They are exceedingly active in warm weather, making short flying excursions from clump to clump of grass. They do not become abundant until mid-April. From the end of April until the end of June they become increasingly difficult to find, but from July onwards there is a second increase and flies continue to emerge until October.

Third-instar larvae may be found throughout the winter in *Lolium italicum*, *L. perenne*, *Dactylis glomerata*, *Poa trivialis*, *P. annua* and wheat. In the areas where these observations were made (Lancashire and East Anglia) tillers with their central shoot withered were very common in clumps of *Lolium perenne*. At the base of these shoots were generally to be found larvae of *Geomyza tripunctata* or *Oscinella frit*. Generally the

percentage of the latter was greater than the former but not infrequently clumps of *Lolium perenne* were examined which contained a larger number of the larvae of *Geomyza tripunctata* than *Oscinella frit*. Generally only about 4–5 % of the tillers were found to be attacked, but in one clump of *Lolium perenne* from a roadside, 16 % of the tillers were killed. Only occasionally were larvae found in the other grasses mentioned above or in wheat.

First- and second-instar larvae were more abundant during May and from August to October than during any other periods of the year. Occasional first-instar larvae were found as late as November and a few second-instar larvae were found in January. Both species overwinter mainly as third-instar larvae and pupate during February and March, unhatched puparia being present throughout the spring and summer. Third-instar larvae, however, occur during any period of the year. From the above observations and from breeding experiments in the laboratory it is concluded that this species probably has two generations per annum.

(*b*) *Laboratory observations*

Experiments were designed to determine the egg-laying habits, the larval host plants and the comparative infestation of various grasses and cereals. Seeds were set in pots in a greenhouse, the cereals and grasses used being wheat, barley, oats, *Lolium perenne*, *L. italicum*, *Dactylis glomerata*, *Poa trivialis*, *Cynosurus cristatus* and *Festuca rubra*. Pots were also sown with mixtures of these seeds. From 5 to 12 days after the plants had appeared above the ground, hurricane-lamp cages containing two male and two female flies were placed over as many pots as possible. (The smallest number of pots of any one cereal or grass over which flies were caged was five.)

Oviposition. Eggs were always laid in the stems of the host plants, generally only one egg per plant. Occasionally, however, two or even three eggs were laid in close proximity to each other. The majority of the eggs were laid at, or just below, soil level, a few higher up the stems, but they were never found above a height of 2 in. When the sheath of the host plant was loose or broken, eggs were always found behind it so that they could not be seen without removing the sheath.

Hatching. In a few instances when hatching was observed the young larva crawled upwards until it reached the base of the leaf blade, when it bored downwards either between the outer sheaths or between a leaf sheath and the central shoot. Examination of these plants showed that the central shoot had been bored into about 2 in. from its base where the

larva had eaten out two or three spiral channels until it arrived at the base of the shoot. It is unlikely that all the newly hatched larvae penetrate to the central shoot in the above manner, for in some instances there was evidence of a more direct penetration.

At the base of the shoot the young larva continued to feed until the central shoot was severed. The damage became more noticeable after about 2 days when the shoot died and turned yellow. After 5–8 days the first instar moulted and the second instar fed for 2-4 days when ecdysis again occurred. In the third instar the larvae fed for 9–15 days. Pupation took place inside the shoot; the pupal period varied from 17 to 30 days.

Emergence from the pupal case is effected by means of the *ptilinium*. This structure is seen to be pushed out and retracted even after the head of the imago has emerged, and the movement continues until the fly is free from the pupal case.

Eggs were found on all the grasses and cereals sown, but on hatching not all of the larvae survived, and although third-instar larvae were found in all cases, there were marked differences in the percentage of plants infested in the different Gramineae. From the number of larvae found and the number of flies hatched out from the various pots it was concluded that the cereals and grasses were preferred in the following order:

(1) *Lolium perenne.*
(2) *L. italicum.*
(3) Wheat.
(4) Barley. *Poa trivialis.* *Dactylis glomerata.*
(5) *Cynosurus cristatus.* Oats.
(6) *Festuca rubra.*

(*c*) *Parasites*

Three species of parasites were bred; these were, *Phaenocarpa livida* Hal., *Chasmodon apterous* Nees, and *Stenomalus* sp. The percentage of parasitized puparia was not determined but the most frequent parasite was *Phaenocarpa livida.*

Of the *Stenomalus* sp. Dr Ferriere states *in litt.*: "This seems to be distinot from *S. muscarum* and is not in the British Museum collection."

III STRUCTURE

(a) *The third-instar larva*

(i) *Morphology.*

The third-instar larva is white and opaque, apodous and amphipneustic. It consists of a head, three thoracic and eight abdominal segments. It varies from 4·0 to 6·6 mm. in length and from 0·8 to 1·2 mm. in width in the widest part, which is that region from the third thoracic to the first abdominal segment. The tapering of the anterior end from this point depends upon the extent of retraction of the head within the first thoracic segment.

The *head* (Fig. 1) is small, rounded anteriorly and tapering posteriorly so that when retracted it fits into the first thoracic segment. When the larva is feeding the

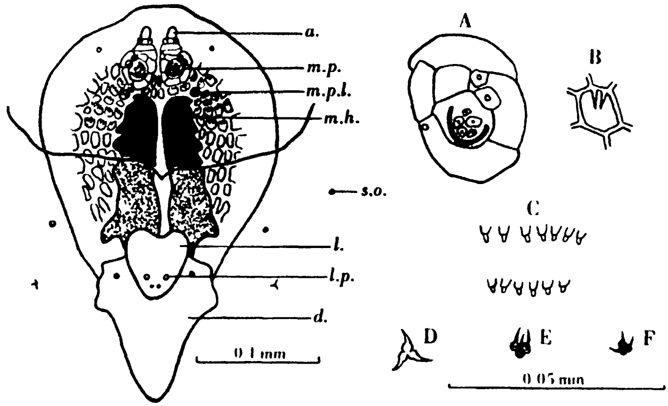

Fig. 1. Ventral view of head of third-instar larva of *G. tripunctata*. *a.* antenna; *m.p.* maxillary palp; *m.p.l.* sensory palp; *m.h.* mouth-hooks; *s.o.* prothoracic sensory organ; *l.* labium; *l.p.* labial palp; *d.* dentate sclerite. A, maxillary palp; B, chitinous teeth; C, thoracic chitinous denticles; D, E and F, vestigial legs of first, second and third thoracic segments respectively.

mouth-hooks are intermittently projected and retracted through the mouth opening which is on the ventral surface of the head, whilst the head itself is extended from and retracted into the first thoracic segment.

The *mouth* opens on the ventral surface of the head immediately beneath the mouth-hooks and between the oral lobes, and leads directly into the pharynx.

The *antennae* (*a.*) are conspicuous, one on either side on the anterior margin of the head. They are two-jointed, having an anterior elipsoidal joint articulating with, and having its base sunk into, the end of the second or basal joint.

The *maxillary palpi* (*m.p.* and *A.*) are situated in front of and below the antennae. Five minute sensory papillae are enclosed in an incomplete chitinous ring, the whole being slightly raised on a short wide basal palp. There are also two small papillae on the outer margin of each maxillary palp immediately anterior to the opening of the chitinous ring, and a pair of minute palpi, one on either side of the head on the inner

side near the maxillary palp. Other cephalic sense organs are a pair of small preoral papillae, one on either side below the maxillary palp, and two minute palpi one on each anterio-lateral margin of the head.

Ventrally the head is almost completely covered with numerous chitinous ridges enclosing polygonal spaces; a number of these ridges have minute chitinous teeth arising from their margins and directed posteriorly as shown in Fig. 1 B. Immediately posterior to the mouth on the ventral surface of the head is a lightly chitinized plate—the *labial plate* or *labium* (*l.*). This bears near the posterior margin two pairs of sensory papillae, the posterior pair being smaller than and placed immediately behind the anterior pair; these are the *labial palpi* (*l.p.*) said by Keilin (1915) to be present in all the cyclorraphous larvae he has examined.

The junction of the body segments is marked by incomplete lines of minute *chitinous denticles* (Fig. 1 C), the arrangement of which has been studied by Mesnil (1935).

Each thoracic segment bears on its ventral surface near its posterior margin a transverse series of six minute sensory organs (*s.o.*) which appear to be sunk in very shallow pits; it also bears on its ventral surface on either side near the ventral line, and immediately posterior to the above sensory organs, a *vestigial leg* (Fig. 1 D). This vestige consists of three minute bristles raised on short basal papillae.

(ii) *The cephalo-pharyngeal skeleton* (Fig. 2).

The *cephalo-pharyngeal skeleton* as compared with that of other acalypterate larvae, is thick and heavily sclerotized. It consists of a pair of *mandibular sclerites* or *mouth hooks* (*m.h.* 3) articulating with an intermediate or *hypostomal sclerite* (*h.*) which is attached to the large *pharyngeal sclerites* (*ph.*). The latter are fused above to a *median dorsal sclerite* (*d.s.*) and below is a median ventral lightly sclerotized plate, the *dentate sclerite* (*d.*).

The mouth hooks articulate with the hypostomal sclerite by means of two slender sclerotized rods. As in the majority of phytophagous larvae the mouth hooks are toothed and move in a median longitudinal plane. The hypostomal sclerites are H-shaped, the articulating rods of the mouth hooks fit into the anterior hollow of the H and the posterior rods of the latter are firmly attached to the pharyngeal sclerites. Each pharyngeal sclerite consists of a single anterior projection and a dorsal and ventral posterior projection. A long thin rod from the anterior projection extends over one of the posterior rods of the hypostomal sclerite. Dorsally the pharyngeal sclerites are fused with and connected by the dorsal sclerite and ventrally are continuous with the base of the pharynx. The dorsal sclerite is not so heavily sclerotized and in the third-instar larva it cannot be detached from the pharyngeal sclerites. The dentate sclerite viewed from the ventral surface is triangular in shape, and from the lateral surface is ≻ shaped, it is very lightly sclerotized and bears two minute prominences on the ventral surface near the anterior margin.

(iii) *The respiratory system.*

The third-instar larva is amphipneustic. The *anterior spiracles* (Fig. 3 A) are very large and prominently placed; they are situated on the dorso-lateral surface of the body near the posterior margin of the pro-thoracic segment. They are particularly broad and are flattened dorso-ventrally, each bearing thirteen to fifteen *digitate processes* (*d.*) which have small openings at their apices. Each opening is surrounded

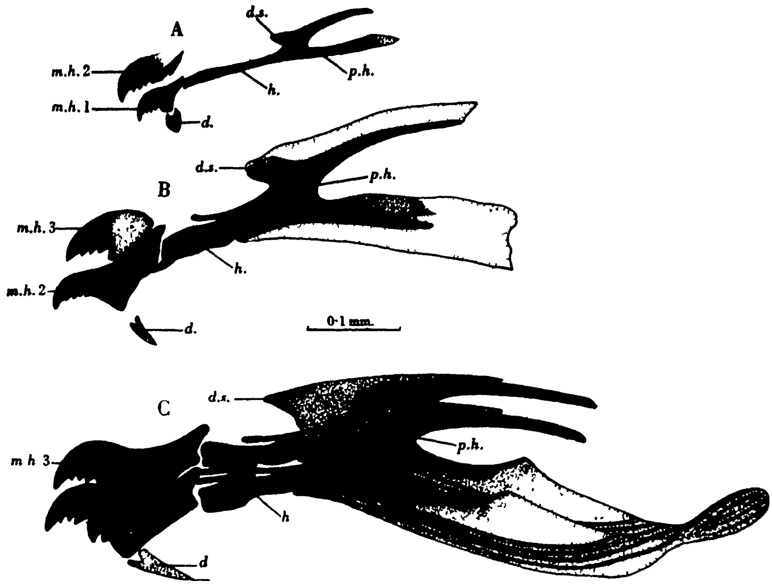

Fig 2. Cephalo-pharyngeal skeletons of the three instars of larva of *G. tripunctata*. A, first instar; B, second instar; C, third instar. *m.h.*1, *m.h.*2, *m.h.*3, mouth-hooks of instars one, two and three respectively; *d.* dentate sclerite; *d.s.* dorsal sclerite; *h.* hypostomal sclerite, *p.h.* pharyngeal sclerite. (In A, *h.* = hypostomal region and *p.h.* = pharyngeal region.)

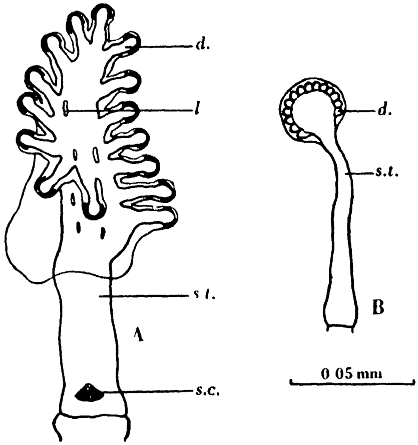

Fig. 3. A and B. Anterior spiracles of first- and second-instar larvae respectively of *G. tripunctata*. *d.* digitate process; *l.* lacuna; *s.t.* stigmatic trunk; *s.c.* stigmatic scar.

by a ring of chitin—the *peritreme*. A series of minute fissures are visible on the main stem of the spiracle which joins on to the short bulbous region of the main tracheal trunk by means of the *stigmatic trunk* or *felted chamber* (*s.t.*). There is a *stigmatic scar* (*s.c.*) on the felted chamber which marks the position at which the second instar larval spiracles became detached on moulting.

The *posterior spiracles* (Fig. 4 A) are situated dorso-laterally on the posterior end of the last abdominal segment; each is borne on the end of a short *stigmatic papilla*. The *stigmatic trunk* of each spiracle opens out on to the *stigmatic plate* (*s.p.*) through three short radiating branches, at the end of each of which is a small circular opening surrounded by a chitinous *peritreme* (*p.*). From the stigmatic plate arise four series of fine *branched chitinous hairs* (*b.h.*) which appear to support a thin membrane.

A main *dorso-lateral longitudinal trunk* (Figs. 4 and 5 B) extends along either side of the body to connect up the anterior with the posterior spiracles of each side.

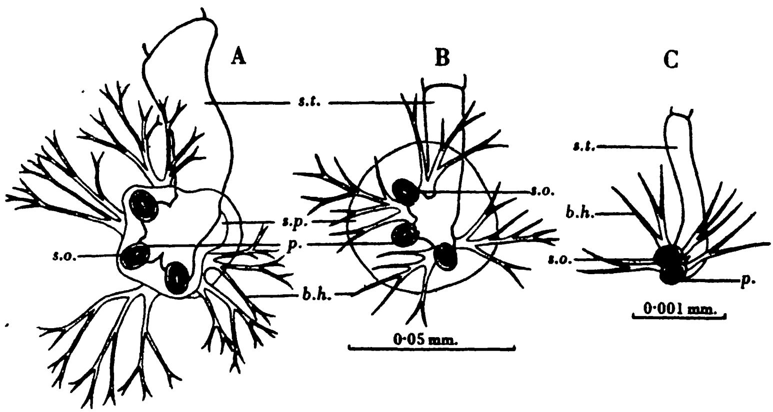

Fig. 4. A, B and C. Posterior spiracles of first, second and third instar larvae respectively of *G. tripunctata*. *b.h.* branched hairs; *p.* peritreme; *s.o.* stigmatic opening; *s.p.* stigmatic plate; *s.t.* stigmatic trunk.

A series of ten *dorsal transverse commissures* (1–10) unite one trunk with the other along the length of the body from the third thoracic to the eighth abdominal segment. The first and last commissures are thickened; the first which is in the anterior region of the third thoracic segment gives off two anteriorly directed branches, one from each side, to the dorsal region of the cephalo-pharyngeal sclerites; the second, in the posterior region of the third thoracic segment, loops forward over the first commissure to supply the posterior dorsal region of the pharyngeal muscles; the third arising in the anterior region of the first abdominal segment extends into the third thoracic segment; the second and third commissures have two unbranched tracheoles extending forward a short distance and arising one on either side near the mid-ventral line. The fourth, fifth, sixth, seventh, eighth and ninth commissures arise from the main trunks at the junction of the segments; the fourth arises at the junction of the first and second abdominal segment and others at each subsequent junction. These commissures loop slightly forward, each has two branched tracheae anteriorly, arising one on either side

near the mid-dorsal line, and two, one on either side directed posteriorly and arising a short distance from the point of junction of the commissures to the main tracheal trunk. The tenth transverse commissure unites the main tracheal trunks immediately behind the junction of the seventh and eighth abdominal segments.

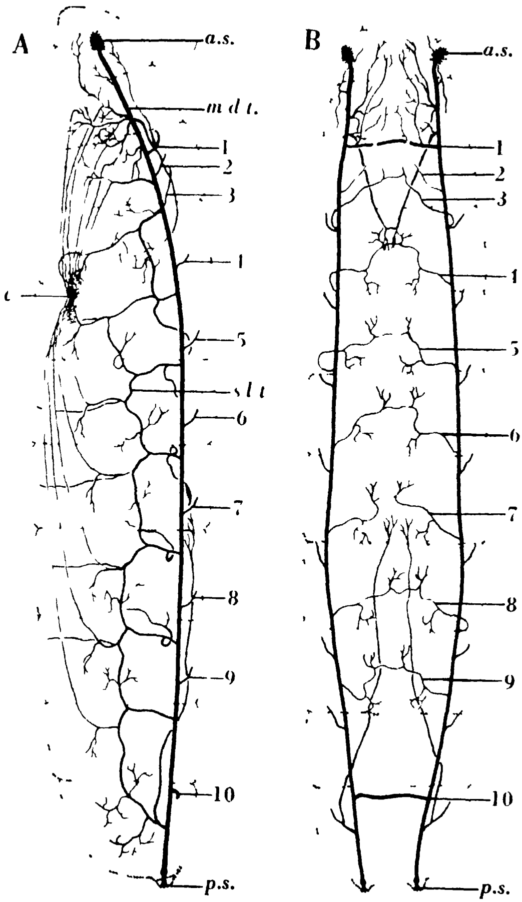

FIG. 5. Respiratory system of third-instar larva of *G. tripunctata*. A, lateral view; B, dorsal view. *a.s.* anterior spiracle, *m.d.t.* main dorsal tracheal trunk; 1–10, dorsal transverse commissures; *c.* concentration of tracheoles at "brain"; *s.l.t.* secondary longitudinal tracheal trunk; *p.s.* posterior spiracle.

The main trunks also give off into each segment a ventral branch. In the thoracic region these branches supply the pharyngeal mass and give rise to five long tracheoles on each side which run posteriorly to supply the brain. In the abdominal segments

the ventral branch subdivides giving an inwardly directed branch to the viscera and a branch which subdivides into a posterior and anterior branch which connect up with the corresponding branches of the adjacent segments to give a *secondary ventral longitudinal trunk* (*s.l.t.*). From this trunk in each of abdominal segments 1–7 a branch is given off which divides to give a branch to the ventral body wall and a branch which runs forward to supply the ventral ganglion (*c.*). The ventral longitudinal trunk also gives off small branches into each segment. The tracheae on either side run forward from the ventral longitudinal trunk to supply the ventral ganglion. After

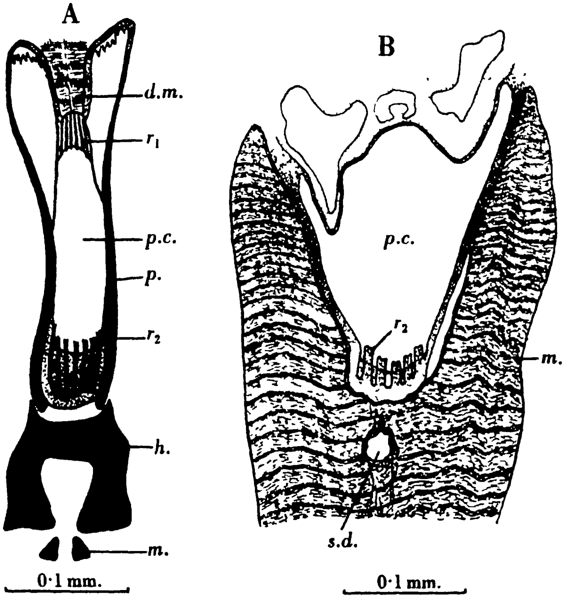

Fig. 6. Transverse sections of pharynx of third-instar larva of *G. tripunctata*. A, near anterior end; B, near posterior end. *d.m.* dilatory muscle; r_1, dorsal longitudinal ridges; *p.c.* pharyngeal cavity; *p.* pharyngeal sclerite; r_2, ventral longitudinal ridges; *h.* hypostomal sclerite; *m.* (in fig. A) mouth-hooks; *m.* (in fig. B) muscle; *s.d.* salivary duct.

entering the ganglion the tracheae subdivide and one tracheole from each branch on one side connects up inside the ganglion with a tracheole from a corresponding branch on the other side.

In the anterior region of the body other tracheae arise to supply the supra-oesophageal ganglion, their number and arrangement can be seen in Fig. 5 A. Six of these tracheae arise in the meso- and metathoracic segments, and one in the first abdominal segment.

(iv) *The pharynx.*

The *pharynx* (Fig. 6) which lies inside and along the base of the cephalo-pharyngeal skeleton, widens gradually from the mouth back to about three-quarters of its length

and then narrows again to join the oesophagus immediately behind the cephalo-pharyngeal skeleton. A series of transverse sections shows that the cavity inside the cephalo-pharyngeal skeleton is roughly oval in outline, and that the pharynx occurs at its trough-like base. Along the bottom of this trough there are six or seven longitudinal grooved ridges (r_2). Keilin (1915) has shown that the presence or absence of these ridges is dependent upon the feeding habits of the larva; that in saprophagous

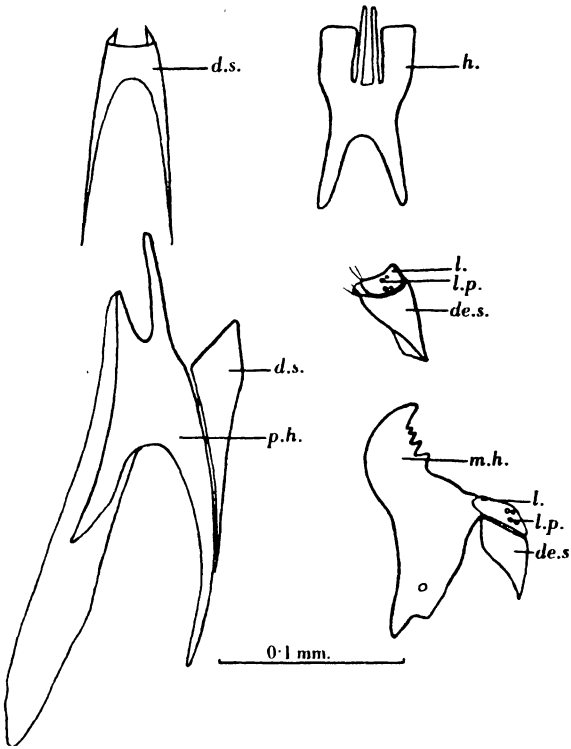

Fig. 7. Parts of cephalo-pharyngeal skeleton of second-instar larva of *G. tripunctata*. *d.s.* dorsal sclerite; *h.* hypostomal sclerite; *p.h.* pharyngeal sclerite; *l.* labium; *l.p.* labial palp; *de.s.* dentate sclerite; *m.h.* mouth-hook.

larvae they are generally well developed and that in phytophagous larvae they are generally absent. He has also shown that intermediate stages occur. This has been observed by Steel (1931) in the larva of *Oscinella frit* where the "arms" of the Y-shaped ridges are slightly longer than those of *Geomyza tripunctata*. Dorsally the pharynx is lined by a cuticular membrane folded into six small longitudinal ridges (r_1), U-shaped in transverse section. Above the cuticular membrane is a hypodermal layer which lines the cavity immediately above the pharynx and extends upwards as far as the insertions of the dilatory muscles. The latter are a pair of muscles which extend

longitudinally above the pharynx having their ventral insertions in its dorsal wall, and their dorsal insertions in the dorsal sclerite of the cephalo-pharyngeal skeleton.

(b) *The second-instar larva*

The second-instar larva is very similar in most respects to the third instar. It varies from 1·8 to 3·5 mm. in length and from 0·3 to 0·8 mm. in diameter in the region of the third thoracic segment.

The *head* and *cephalic sense organs* are miniatures of those of the third-instar larva. The vestigial thoracic legs (Fig. 1 E) and the thoracic sense organs are also homologous with, but smaller than, those of the third instar. The denticles on the thoracic segments are larger than those on the abdominal segments, those on the prothoracic segment being slightly larger than those on the meso- and metathoracic segments.

The number of component sclerites in the *cephalo-pharyngeal skeleton* (Fig. 7) is the same as in the third instar, all the sclerites are smaller but similar in shape. There is a pair of *mouth-hooks* (*m.h.*), a *hypostomal sclerite* (*h.*), a pair of *pharyngeal sclerites* (*ph.*), a *dorsal sclerite* (*d.s.*) and a *median ventral* or *dentate sclerite* (*de.s.*). Each mouth-hook differs slightly in shape from that of the third instar and is comparatively longer and less deep. It has a large apical tooth and four smaller ones which vary slightly in size and shape in different larvae. The teeth may be almost equal in size or the two posterior ones may be slightly larger.

The larva is amphipneustic and, except in the structure of the anterior spiracles, the respiratory system is almost identical with that of the third instar. Each *posterior spiracle* (Fig. 4 B) opens out through three *spiracular clefts* (*s.l.*) on a *stigmatic plate* (*s.p.*) at the end of a short papilla on the last abdominal segment. From the plate arise four series of *branched hairs* (*b.h.*) as in the third instar.

The *anterior spiracles* (Fig. 3 B) open out on the prothoracic segment and each has a comparatively long *stigmatic trunk* (*s.t.*), the number of spiracular clefts corresponds with the number in the subsequent third-instar larva. In this instar, however, the openings are situated at the ends of very short *digitate processes* (*d.*), each process being closely applied to its neighbour to form the major sector of a circle and the whole having the appearance of a transverse section through a rosette.

(c) *The first-instar larva*

The first-instar larva is very small, comparatively narrower and more translucent. Immediately after hatching it is about 0·9 mm. long and 0·17 mm. broad, but just before ecdysis it may attain a length of 1·8 mm. and a breadth of 0·3 mm.

The *head* (Fig. 8) is comparatively large, with a median ventral depression, the cephalic sense organs are homologous with those of the second and third instars. The *two-jointed antennae* (*a.*) are prominently placed on the anterior surface, the *maxillary palpi* (*m.p.*) are in a more ventral position and there are also a number of smaller *sensory papillae* (*s.p.*). The *labium* (*l.*) is lightly chitinized and bears a pair of minute papillae, the *labial palpi*, one on either side. There are no ridges on the ventral surface of the head similar to those described in the third instar. The *cephalic sensory organs* and *vestigial legs* are smaller but homologous with those of the second- and third-instar larvae. There are chitinous denticles at the junction of the segments as in the second- and third-instar larvae.

The *cephalo-pharyngeal skeleton* (Figs. 2 A and 8) is more simple than that of the second and third instars. Viewed from the lateral surface, only three sclerites can be distinguished—a *mouth-hook* (*m.h.*), a *median ventral* or *dentate sclerite* (*d.*) and a *pharyngeal sclerite* (*ph.*) termed by Keilin (1915) in the first-instar larva of *Pollenia rudis* "la pièce basilaire". The latter is not composed of two sclerites (the hypostomal sclerite and pharyngeal sclerite), as in the second and third instars, but consists of an anterior projection and two posterior projections. They are fused to one another only in the anterior dorsal region where there is a projection from either side to form a rudimentary *dorsal sclerite* (*d.s.*). Each mouth-hook has a large apical tooth and a series of small indentations. There is also an accessory sclerite (*a.s.*) at its base.

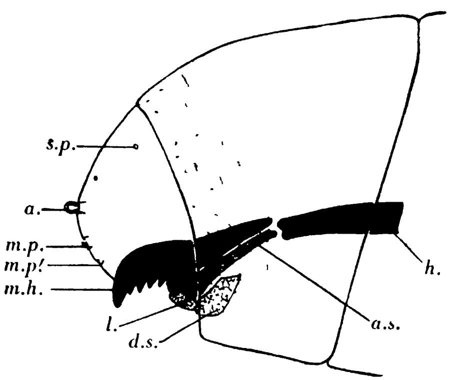

Fig. 8. Head of first-instar larva of *G. tripunctata*. *s.p.* sensory papilla; *a.* antenna; *m.p.* maxillary palp; *m.p.'* smaller palp; *m.h.* mouth-hook; *l.* labium; *d.s.* dentate sclerite; *a.s.* accessory sclerite; *h.* hypostomal region.

The first-instar larva is metapneustic as are the other first-instar larvae of cyclorraphous Diptera examined during the present investigation (Thomas, 1933, 1934). In the first instar larva of *Chlorops taeniopus*, however, Frew (1923) has observed that although hairs are present on the papillae of the last abdominal segment "these show no trace of stigmatic openings". Except for absence of anterior spiracles the general plan of the tracheal system in this instar is the same as that of the second and third-instar larvae. Each *posterior spiracle* (Fig. 4 C) has two *stigmatic openings* (*s.o.*), placed very near together at the end of a short *stigmatic papilla*; each opening is surrounded by a chitinous *peritreme* (*p.*) near which the *branched hairs* (*b.h.*) arise. There is a long *stigmatic trunk* (*s.t.*) which gives rise to a *main dorsal tracheal trunk*. In the region of the pro- and mesothoracic segments each trunk divides into tracheoles which supply the head and thoracic segments. There is a concentration of tracheoles in the region of the "brain" but this is not nearly as marked as in the second- and third-instar larvae.

(*d*) *The egg*

The egg (Fig. 9) varies in length from about 0·85 mm. when laid, to about 1·10 mm. immediately before hatching, and is about 0·2 mm. in diameter. It is fusiform in shape, slightly flattened on its ventral surface and more broadly rounded at the posterior than at the anterior end. It is white and glistening and its surface is marked by a series of longitudinal ridges and grooves most of which extend from one end of the egg to the other but occasional ridges end abruptly. The whole surface is covered by a series of minute papillae of thickened chorion, which are more numerous in the ridges than in the grooves. The anterior or *micropylar area* (*m.*) is considerably thickened and drawn out in the form of a plug of hardened chorion.

Fig. 9. Egg of *G. tripunctata*. *m.* micropyle.

(*e*) *The puparium*

The puparium (Fig. 10) is formed of the hardened integument of the third-instar larva. It is dark brown in colour and varies in length from 3·6 to 4·0 mm. with an average maximum breadth of 1·0 mm. It is approximately cylindrical in shape, the segments being homologous with those of the third instar. The thoracic segments are flattened on the dorsal surface which forms an angle of about 45° with the ventral surface. The larval head is completely retracted within the prothoracic segment, the larval spiracles (*a.s.*) remain protruding from the anterior end of the puparium. The posterior end is very wrinkled, the larval anus is present on the ventral surface of the last abdominal segment and the larval stigmatic papillae (*s.p.*) project from its posterior end.

The cephalo-pharyngeal skeleton of the larva remains inside the puparium fused into the ventral surface of the thoracic segments.

When the adult emerges, a slit is formed ventral to the edge of the sloping portion of the thoracic segments, this slit extends posteriorly as far as the anterior end of the first abdominal segment; in this region a horizontal slit occurs on either side, the two slits may meet dorsally when the flat portion of the thoracic segments becomes detached. The horizontal slit may also extend a considerable distance around the ventral half of the puparium so that the portion comprising the ventral half of the thoracic segments may be partially detached.

IV. Summary

In the field the chief larval host plants of *Geomyza tripunctata* Fall. are *Lolium perenne* and *L. italicum*; in the laboratory other grasses and wheat are readily attacked. The damage is similar to that caused by *Oscinella frit* L.; the larva feeds inside the grasses and kills the central shoot.

Adults emerge in March and April and there are two generations per annum; the species overwinters in the larval stage and pupation takes place inside the host plant near the base of the shoot.

The egg, the three larval instars, and the puparium are described.

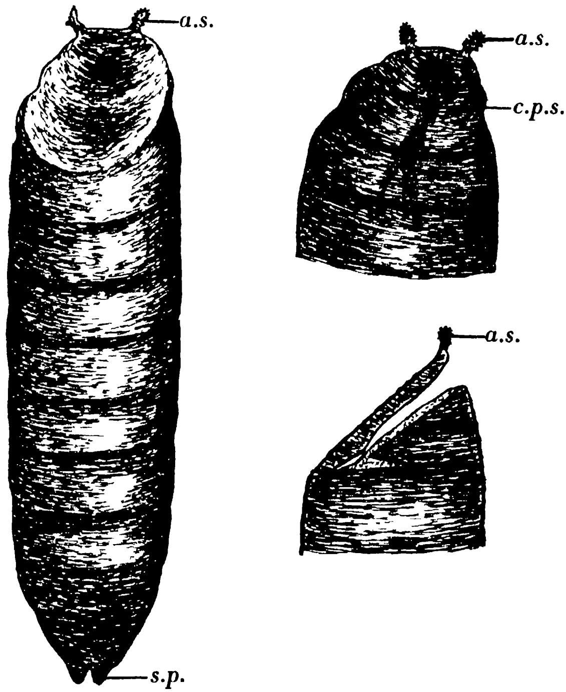

Fig. 10. Puparium of *G. tripunctata*. *a.s.* anterior spiracle (of larva); *c.p.s.* cephalo-pharyngeal skeleton; *s.p.* stigmatic papilla.

The writer is indebted to Dr H. W. Miles for help and advice during the early stages of the investigation when he was a Grisedale Scholar at the Victoria University, Manchester. He is also indebted to Mr F. R. Petherbridge for helpful criticism when the work was continued at the School of Agriculture, Cambridge.

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FIELD INVESTIGATIONS UPON THE CONTROL OF THE MUSTARD BEETLE, *PHAEDON COCHLEARIAE* F., ON WATERCRESS

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(With Plate IV)

CONTENTS

I. INTRODUCTION

DURING recent years, *Phaedon cochleariae* has been abnormally abundant on watercress in Glamorganshire, Monmouthshire, and many other parts of Britain. Attacks of great severity were reported during 1935 and 1936, and watercress was so extensively damaged in some instances that entire loss of crop resulted.

Most of the information hitherto available concerning control methods is contained in the Bulletin on Salad Crops and in Advisory Leaflet No. 157, published by the Ministry of Agriculture and Fisheries (1936*a*, *b*). The recommendation is made that in localities liable to infestation all watercourses and the sides of watercress beds, especially if no concrete work is employed, should be maintained in a clean condition, in order to decrease the number of places suitable for the hibernation of the adult beetles. For the purpose of dealing with attacks as they arise in summer, flooding of the affected beds with water for a few hours, where possible, is suggested since this would tend to drown the adult and larval stages

or sweep them away from the beds. There is also a brief reference in the leaflet to the use of derris or pyrethrum washes.

In 1931 pyrethrum and derris preparations were included by Thompson (1932) in preliminary field trials designed to discover a non-arsenical insecticide which would be sufficiently toxic to destroy the pest but which would not contaminate the watercress in any way or affect its market value. Both types of preparations were applied in the form of a spray on six occasions at intervals of a week, and the derris was also tested as a dust. After a preliminary trial the use of derris dust was, however, discontinued on account of the large amount required to produce satisfactory results and the high cost of the material. Both the pyrethrum and the derris washes were considered effective as a control measure though it was not found possible to estimate the actual percentage of beetles killed owing to the dense foliage of the watercress. The percentage of the active constituents present in the washes used in these trials is not stated.

Experience during the summer of 1934 showed that, apart from attention to sites which might provide winter shelter to the adult beetles, none of the measures advised for the control of *P. cochleariae* were practised by the commercial watercress growers in the counties of Glamorgan and Monmouth since these had been found unsatisfactory. Thus flooding of affected beds with the object of drowning or sweeping away the adults and larvae had been found only partially effective, probably because upon the rising of the water level a high percentage of the beetles invariably leave the watercress beds for the adjoining banks and are, in any case, capable of remaining afloat on the surface of water for a long time without being seriously affected. Further, watercress beds are generally low-lying with the result that the normal level of the water closely approximates the tops of the banks and the flow of water through the beds is usually too sluggish to wash away a large proportion of the insects. Pyrethrum had been tried by some growers as a spray fluid in the summer months, while others had relied for a time upon frequent applications of derris washes. The degree of control obtained by these methods had apparently been exceedingly inconstant and, on the whole, disappointing. This may perhaps be explained by the fact that both these insecticides suffer from the disadvantage of being variable and unstable substances and that, hitherto, no information has been available concerning the minimum potent concentration of the toxic constituent in the diluted solution of these products necessary to give the desired results.

The experiments described in the present communication were undertaken to investigate further the toxicity of derris and pyrethrum preparations to *P. cochleariae* on watercress, and to study the phytocidal effects of these products upon the plant itself.

II. General arrangement of the experiments

The experiments were carried out in 1935 at two different centres and in each case on watercress beds where attacks of great severity had occurred in the preceding two seasons. The results obtained at only one of these centres is discussed fully in the present paper, since the experiments at this centre were the more comprehensive and are essentially similar to those at the other centre. The experimental area consisted of 15 beds or plots (Pl. IV, fig. 1), each of which had an area of approximately 160 sq. yd. A group of three plots was taken as a unit for any one treatment. Four distinct preparations were tested and one group of plots retained as untreated controls. The treatments applied to the respective plots were as follows:

Plots 1*a*, 1*b*, 1*c*: Control (untreated).

Plots 2*a*, 2*b*, 2*c*: Derris wash with sulphonated lorol incorporated as a spreader, the wash being made up to contain 0·004 % rotenone and 0·05 % sulphonated lorol.

Plots 3*a*, 3*b*, 3*c*: Derris dust "A" having a rotenone content of 0·2 %.

Plots 4*a*, 4*b*, 4*c*: Derris dust "B" having a rotenone content of 0·5 %.

Plots 5*a*, 5*b*, 5*c*: Pyrethrum emulsion containing 0·01 % of pyrethrin 1.

The following diagram shows the arrangement of the plots together with the nature of the treatment applied to each plot:

Plan of experiment

2*a*	3*a*	1*a*	4*a*	5*a*	3*b*	2*b*	1*b*	5*b*	4*b*	2*c*	3*c*	1*c*	5*c*	4*c*

III. Application of the insecticides

By the end of June an enormous population of *P. cochleariae* had established itself upon the watercress beds, many of the plants bordering the banks being eaten to such an extent that little but the stems and the mid-ribs remained. Eggs, larvae and adults were present and as far as could be judged visually, the infestation was of uniform intensity over all the plots throughout the experimental area. Two applications of each

insecticide were given, the first on 24 June and the second on 5 July. The weather on each of these dates was very warm, sunny and calm except for an occasional slight breeze. Drift of the different preparations to neighbouring plots was avoided by the careful use of a hessian screen. No rain fell during the period 24 June to 10 July when the counts were made of the number of live beetles present on all the treated and control plots.

The machines used for the purpose were a pneumatic knapsack sprayer for the washes and a rotary hand blower for the dusts. Both spray fluids were applied at the same pressure and in such a way that the entire foliage was thoroughly wetted. The two dusts proved easy to handle and gave an excellent cover. Approximately equal amounts of each were utilized on the respective plots, though there was necessarily some variation between the plots even under the same treatment on account of slight differences in the size of the plants. In all cases sufficient dust was used to cover the whole of the foliage satisfactorily.

Application of all the insecticides on both occasions was done personally by the writer so as to ensure uniformity of treatment. The applications were aimed at killing the maximum number of the adults and larvae, and therefore all of them were made during the sunniest part of the day when the greatest number of beetles collected on the upper surface of the leaves.

IV. Estimation of results

Some 4 or 5 days after the second application of the insecticides, estimations were made of the actual number of live beetles present on both treated and control plots. The technique adopted consisted in counting all the live beetles that could be seen on all the plants within areas of 1 sq. ft., ten random areas being taken as the unit for the determination of the beetle population on any one plot. Each square foot area was defined by a wooden quadrate placed in position on the day prior to that on which the second application of the treatments was made.

Care was exercised when the counts were made to stand a short distance away from the area under examination and facing the sun so that no shadow was cast on the plants, since the beetles immediately drop and feign death when disturbed. Further, all the counts were conducted during the hottest hours around midday, the period when the beetles are most active and the majority feeding on the dorsal side of the leaves near the crown or the growing point of the plant.

The relative index figures of the beetle population per square foot on both treated and untreated plots are recorded in Table I.

V. Discussion of results

From an examination of the figures for the beetle population in Table I, it is evident that the degree of infestation was of a fairly uniform order over the area covered by the plots judging by the number of beetles present on the different control plots (Nos. 1*a*, 1*b*, 1*c*). A further striking feature is the very high population of beetles throughout the experimental area. The average number for the three control plots was 322 to the square foot or approximately 14 million per acre.

Table I

Relative index figure of the beetle population after treatment

Treatment	Index of plot	Number of beetles per 1 sq. ft.	Average % decrease of beetles
Control (untreated)	1*a*	311	
	1*b*	332	Nil
	1*c*	323	
Pyrethrum wash	2*a*	121	
	2*b*	131	62·1
	2*c*	114	
Derris wash	3*a*	81	
	3*b*	63	77·6
	3*c*	72	
Derris dust "A" (0·2% rotenone)	4*a*	37	
	4*b*	44	86·3
	4*c*	51	
Derris dust "B" (0·5% rotenone)	5*a*	10	
	5*b*	12	96·9
	5*c*	8	

The figures for the average percentage reduction in the beetle population, shown in the final column of Table I, indicate that all the insecticides tested had been used with decided advantage. The greatest reduction is found on the plots treated with derris dust of 0·5% rotenone content (plots 5*a* 5*b*, 5*c*). The average number of beetles on these three plots was 10 per sq. ft. compared with 322 for an equal area on the controls (plots 1*a*, 1*b*, 1*c*) showing a decrease in infestation due to this treatment averaging 96·9%. A reduction of such a heavy infestation to a figure in no instance greater than 3·7% (plot 5*b*) by dusting the watercress plants on two occasions is considered highly satisfactory from a practical standpoint. Immediately after the dust had been applied on both occasions, a large number of beetles and larvae were found floating on the surface of the water under the dusted plants, definitely affected

by the dust. Observations on the day following treatment showed that all the beetles and larvae were dead except for a few isolated adults which appeared entirely free from any deposits of the dust on their bodies. Moreover, the plants on the plots which received this treatment did not again become appreciably infested whereas on the controls the beetle population continued to develop and the plants were so badly attacked as to be quite worthless and unmarketable. The photograph on Pl. IV (fig. 2) was taken during the third week in July, that is, a fortnight after the second application of the insecticides, and shows the appearance of a typical plant on the left from one of the plots (plot 5*a*) dusted with derris of 0·5 % rotenone content and, on the right a plant from one of the controls (plot 1*a*). In order to safeguard against the movement of beetles to the treated plots, all the controls were dusted in the latter part of July with this derris preparation.

The next most efficacious insecticide was derris dust of 0·2 % rotenone content (plots 4*a*, 4*b*, 4*c*). The two applications of this dust had reduced the average number of beetles to 44 per sq. ft., a decrease of 86·3 % by comparison with the infestation on the untreated plots. When this dust was used it apparently destroyed all the beetles that were touched by it, but subsequent observations revealed that many of the supposedly dead beetles recovered some time afterwards.

The derris wash also showed a marked toxicity towards the beetles at the concentration tested (plots 3*a*, 3*b*, 3*c*). The intensity of infestation compared with that on the controls had been reduced by this treatment from an average of 322 to 72 beetles per sq. ft. The percentage mortality caused by this treatment was therefore at the rate of 77·6 compared with 86·3 and 96·9 for the two derris dusts. The limitation of the degree of control given by the derris wash was probably due at least in some measure to the low, dense foliage produced by the watercress plant and, in consequence, the impossibility of applying the spray in such a manner as to reach all the beetles and the larvae present on the undersides of the leaves. It was also found in later observations, as in the case of the derris dust of 0·2 % rotenone content, that many of the beetles which at first appeared dead were only temporarily paralysed.

The pyrethrum wash afforded some considerable protection against the beetle, but did not, in the circumstances described, prove of equal value to the derris preparations. The number of beetles found after treatment on the plots which received this insecticide (plots 2*a*, 2*b*, 2*c*) represented about a third of the infestation on the controls (plots 1*a*, 1*b*, 1*c*). Under the most favourable conditions, with very thorough and

careful application, the pyrethrum wash killed 62·1% of the beetles, whereas the derris wash showed a toxicity of 77·6%. No definite conclusions can be safely drawn as to the reasons for the failure of the pyrethrum wash to exert a control of the beetles at least equal to that derived from the use of the derris spray fluid but it may be pointed out that such failure might have been due to the difference detected in the wetting properties of the two emulsions rather than to any difference in the insecticidal potency of their respective toxic constituents. The spreading power of the derris preparation was definitely superior to that containing the pyrethrum extract.

VI. Difficulties involved in the general application of insecticides in commercial culture of watercress

Great practical difficulties are involved in applying insecticides in the field for the successful control of *P. cochleariae* on watercress plants. The use of arsenicals is open to serious objection on account of the difficulty in ensuring, even with thorough washing of the plants, that all traces of arsenic are removed before marketing the crop. The watercress grower is thus driven to other insecticides and from a consideration of the data presented in this paper it would seem that an efficient control can be secured by means of derris preparations which do not possess the poisonous properties of those containing arsenic. It must, however, be borne in mind that serious consequences may follow the indiscriminate use of derris or other insecticides containing rotenone since they are highly toxic to fish and other cold-blooded animals as well as to insects. They should on no account be used in circumstances in which there is any risk that the liquid or dust, even in very small quantities, might reach rivers, ponds or streams containing fish.

In addition to the danger of poisoning fish, it is realized that it would be unavoidable, in commercial practice, for some of the beetles sheltering on the undersides of the leaves or low down on the stems to escape the effects of such insecticides on account of the dense nature of the foliage produced by the watercress plant. For this reason several applications might become necessary in order to secure a satisfactory measure of control, the number being naturally dependent on the thoroughness of applications made and on the climatic conditions prevailing at the time of treatment. In bright sunny weather the beetles are exceedingly active, clustering in enormous numbers for feeding and mating purposes on the upper surface of the foliage in the region of the growing point or crown of the plant, whereas in dull weather they hide and comparatively

few are seen. Moreover, experience with contact insecticides in the control of other pests on different crops indicates that the penetrating and wetting properties of the wash would also have an important bearing on the number of applications necessary to give the desired results. Finally, in view of the overlapping nature of the life cycle and the fact that the insect is only vulnerable in the adult and larval stages, it follows that even the most successful treatment applied under the most favourable conditions would have to be repeated at definite intervals.

VII. Practical conclusions

In view of the observations recorded during 1936 from several centres in South Wales and Monmouthshire where watercress growers had tried out a schedule of direct control measures based upon a consideration of the results obtained from the 1935 field experiments described in this paper and from a study of the life history of the beetle, it is evident that attacks can for all practical purposes be suppressed by the judicious use of derris dusts containing not less than 0·2 % rotenone, if the conditions are such that there is no risk of poisoning fish. The dusts should be applied whenever possible in bright sunny weather, preferably during the hottest hours around midday, when the beetles are at the height of their activity and the majority on the upper surface of the foliage. This treatment when properly carried out will destroy both the adult and larval stages, but it is found desirable in commercial practice to examine the plants carefully some 24 hr. after application for the presence of beetles, and if numerous a further dusting should be given without delay. Applications of these dusts should be repeated after an interval of approximately 10 days in order to kill the larvae and adults which may have emerged since the first treatment, before the latter have attained maturity.

VIII. Summary

1. An account is given of field experiments in 1935 with pyrethrum and derris preparations for the control of *Phaedon cochleariae* F. on watercress plants.

2. Average infestations, judging by the beetle population on the untreated plots, were reduced by:

(*a*) Pyrethrum emulsion of 0·01 % content of pyrethrin I to 37·9 %.
(*b*) Derris wash of 0·004 % rotenone content to 22·4 %.
(*c*) Derris dust of 0·2 % rotenone content to 13·7 %.
(*d*) Derris dust of 0·5 % rotenone content to 3·1 %.

Fig. 1

Fig. 2.

EDWARDS.—Field Investigations upon the Control of the Mustard Beetle, *Phaedon cochleariae* F., on Watercress (pp. 197–205)

3. The difficulties involved in the general application of insecticides for the successful control of the beetle in commercial culture of watercress are discussed.

4. A basis for controlling the beetle by means of direct measures is presented in the light of observations on extended trials by watercress growers in 1936, based upon a consideration of the results obtained from the 1935 field experiments and from a study of the life history of the insect.

Grateful acknowledgements are due to Dr H. Martin, of the Long Ashton Horticultural Station, for kindly determining the pyrethrin content of a sample of the pyrethrum preparation used in the 1935 field experiments described in this paper, and to the watercress growers in the counties of Glamorgan and Monmouth, particularly Mr W. Lewis, Llanmartin, Newport, for their helpful co-operation.

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EXPLANATION OF PLATE IV

Fig. 1. The general lay-out of the watercress beds at the principal centre involved in the field experiments upon the control of *Phaedon cochleariae* F. (see p. 202).

Fig. 2. Character of the damage caused by *Phaedon cochleariae* F. to watercress plants and the result of dusting with derris of a high rotenone content. Typical specimen, on the left from a plot dusted with derris containing 0·5 % rotenone and, on the right from one of the controls (see p. 202).

(*Received* 18 *June* 1937)

NOTES

OBSERVATIONS ON PEAR SCAB (*VENTURIA PIRINA* ADERH.)

By W. F. CHEAL, D.I.C., N.D.A.

AND

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(With Plate V)

In 1932 the writers commenced independent observations on the biology of the apple scab fungus (*Venturia inaequalis*) and the pear scab fungus (*Venturia pirina*) and, later, separate accounts of these were given (Cheal, 1933; Dillon Weston & Petherbridge, 1933). The following observations concern only pear scab and were made for the greater part in Cambridgeshire.

Observations on initial infection

One of us (Dillon Weston, 1933) had already determined by means of spore-trapping experiments that the expanding buds of Doyenne du Comice and Durondeau were infected by conidia from wood pustules before there was any apparent discharge of ascospores. It was of interest therefore to determine more precisely when this infection took place. Some pear trees in a private garden at Cambridge were kept under observation and it was noted that, on several of the scabbed twigs, the fungus stroma was exposed as early as February. Similar records have been made by Cheal (1933), Marsh (1933) and Salmon & Ware (1937).

In 1936 a pear tree which was wood susceptible was kept under daily observation and on occasions was examined during and after showers of rain. The small droplets which hung from some of the buds after the rainstorms were examined microscopically and found to contain conidia. At later stages of development when the buds were bursting, and also when the young rolled leaves were appearing, minute glass capillary tubes were inserted in these hanging drops and the water was examined. The samples which were taken contained conidia. It seemed clear, therefore, that many of these buds were literally bathed in spores and that when the water evaporated the spores were either left on the bud scales or, depending upon the degree of bud development, slightly drawn up amongst the young leaves and developing flowers. It was thought that if infection took place at this early stage it must follow that the under surfaces of the leaves and the calyces would first become infected. Observation showed that this happened. These records have a direct bearing on control measures and further support the conclusions arrived at by Marsh (1933) who states: "A longer programme of sprayings than for the apple is inevitable, and when pear trees are heavily infected with scab, much more than a single pre-blossom and a single post-blossom spraying is necessary."

The source of the inoculum which causes infection

In March 1933 three-year-old Fertility shoots were obtained from an orchard at Houghton in Huntingdonshire and it was observed that there were scab pustules on the one-year-old wood, none on the second, but that on the third-year wood the bark showed superficial cankers and had the appearance of having been badly attacked by scab. Since spores were noted here germination tests were made to see if they were viable and it was found that the germination percentage after 10 hr. was 1, as compared with 64 with the spores from the one-year-old wood. A similar observation was made on the variety Williams' Bon Chrétien but, in this case, no germination was recorded from spores on the three-year-old wood. It was then wrongly inferred that the spores which germinated from this three-year-old wood were spores which had been washed down from the viable pustules on the one-year-old wood.

One of us (Cheal, 1933) had drawn attention to the fact that Prof. E. S. Salmon had stated but not recorded that *Venturia pirina* persisted on two-year as well as one-year-old wood. As we could find no detailed observations on the production of viable spores from pustules on such wood the writers commenced a field investigation in the winter of 1936–7. Scab pustules were soon found on two-year-old wood but the pustules were cut off by cork barriers and flaked off easily when they were subjected to slight pressure, especially on young vigorous trees. On certain trees complete abscission of scab infected tissue was observed even on one-year-old wood.

It was in a seven acre plantation of twenty-year-old Conference trees at Tydd Gote that scab pustules producing viable spores on two-year-old wood were first found. It is of interest to record here that the fruit in this orchard had been badly blemished by scab for a number of years, since it is generally considered that this variety is one which is relatively resistant (Pl. V, fig. 1).

As it was established that scab pustules were present on two-year-old wood we made more detailed examination of older wood and found that in some cases this too was attacked. Pustules were noted on three-year-old wood on the Conference trees at Tydd Gote, on Pitmaston Duchesse in a private garden at West Malling, Kent, on Doyenne du Comice in a commercial plantation at Wilburton, and on an unknown variety in a private garden at Girton, Cambridge (Pl. V, figs. 2 and 3). The latter was a very old tree, suffered badly from scab and as far as is known had never been sprayed. Surface cankers on the wood were very apparent and, when these were further examined, it was seen that scab pustules were present on the edges of many of them and in some cases on four, five, six, seven and eight-year-old wood (Pl. V, fig. 4). We germinated the spores from some of these and found that they were viable. It would seem, therefore, that pustules of pear scab on wood older than one year are not uncommon, and it is inferred that in some cases, as yet not definitely explained, the fungus evades the cork barrier and so becomes perennial.

Control measures

It is suggested that these observations have a direct bearing on control measures and indicate further the importance of pre-blossom fungicide sprays and also their thorough application to older wood.

Acknowledgements

We are indebted to Prof. F. T. Brooks, F.R.S., The Botany School, Cambridge, for kindly confirming the presence of scab pustules on one of these older pear branches.

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EXPLANATION OF PLATE V

Fig. 1. Badly scabbed peduncle of Conference pear.

Fig. 2. Two-year-old branch of Doyenne du Comice showing scab pustules on the wood.

Fig. 3. Three-year-old branch of Doyenne du Comice showing scab pustules on the wood.

Fig. 4. Eight-year-old pear branch. Conidial pustules present at point indicated by the pins.

(*Received* 3 *August* 1937)

Fig. 1.

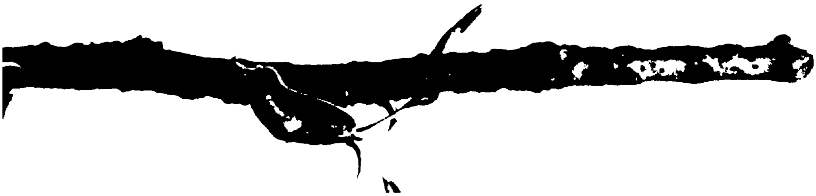

Fig. 2

Fig. 3.

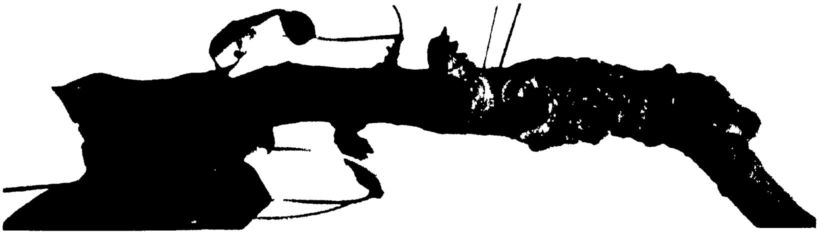

Fig. 4.

A FIELD OBSERVATION ON *OPHIOBOLUS GRAMINIS*

By W. A. R. DILLON WESTON
School of Agriculture, Cambridge

In the past four years several interesting cases of wheat or barley failures in Norfolk have been investigated. At or just prior to harvest, enquiries have been received concerning the cause of the thin stands, empty bleached ears, and prematurely ripened grain that are the features of such failures. The observations made suggest that, in many cases, the major predisposing factor leading to the condition is the taking of corn crops too frequently—or ill-advisedly—in the rotation, and that the chief cause of the trouble is the take-all fungus, *Ophiobolus graminis* and sometimes, in addition, wheat stem Sawfly, *Cephus pygmaeus* or Hessian fly, *Mayetiola destructor*. It is not the purpose here, however, to discuss the symptom complex of such failures or the pathogens concerned but to record an interesting case of a barley failure which was brought to my notice by Mr D. H. Findlay, of the Department of Agricultural Education, Norfolk. On a farm in 1933 a 14-acre field was sown with barley and in 1934, with sugar beet, the tops being ploughed in. Wheat was then taken on half of the field and oats on the other half and, in 1936, the whole field was drilled with barley. The manurial treatments per acre were as follows: 1933, 2 tons $CaCO_3$; 1934, farmyard manure and 4 cwt. superphosphate of lime; 1935, 3 cwt. superphosphate of lime; 1936, 1 cwt. sulphate of ammonia. The soil was light and sandy and of glacial origin. The pH of the top soil was 7·2; it contained 0·07% free $CaCO_3$ and the citric soluble P_2O_5 and K_2O were 0·018 and 0·006% respectively. These figures for citric soluble phosphate and potash are rather below the average for land of that type in that district and suggest a slightly subnormal level of fertility but no serious deficiency. In 1936, in the barley which followed that half of the field taken with wheat, 60% approximately of the tillers showed infection with *Ophiobolus* and 5% had been attacked by the Hessian fly. These facts were reflected in the yield, since the barley after the wheat was assessed at half a sack to the acre whereas, after the oats, it was estimated as yielding between 6 and 7 sacks per acre.

Garrett (1936) in a recent paper says: "*Ophiobolus graminis* can spread through the soil only along the roots of its host plant. A distinction can thus be made between two phases in the activity of the fungus, a parasitic or ascendant phase, in which the fungus is actively increasing on the roots, and a pseudo-saprophytic or declining phase, in which the fungus is merely persisting in dead host tissue. In the declining phase, the disappearance of the fungus from the soil must be hastened by the action of the soil saprophytes in actually decomposing its mycelium." It is of interest to speculate on the source of the infection on this particular field in Norfolk. That the disease was caused in 1936 by wind-borne ascospores from possible neighbouring sources is unlikely since, in that case, it would be reasonable to assume that the crop would be more or less uniformly infected. This was not the case. It may be, however, that in the previous year the half of the field sown with wheat was infected in this

way and that the pseudo-saprophytic or declining phase persisted on the dead roots until the barley was drilled.

An alternative possibility is that the 1933 barley crop was infected and that the declining phase lingered over in the soil to the autumn of 1935, when it was resuscitated by meeting wheat roots and so commenced again as ascendant parasitic phase. On the other half of the field, however, not wheat but oats was sown and the fungus was not able to survive on the roots of this host, consequently the barley that followed the oats was a successful crop, whereas the barley that followed the wheat was a failure.

The writer is grateful to Mr D. H. Findlay, the Department of Agricultural Education, Norfolk, for bringing this case to his notice and also to Mr F. Hanley, the School of Agriculture, Cambridge, for his analysis and comments on a soil sample that was taken from the field.

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(*Received* 22 *September* 1937)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

ORDINARY MEETING of the Association held on Friday, 8 October 1937, at the Imperial College of Science and Technology, London. The Chair was taken at 11.45 a.m. by Mr C. T. GIMINGHAM (Vice-President) and at 2.30 p.m. by Dr J. HENDERSON SMITH (President).

The following papers were read:

At the Morning Session:

I. The Wireworm Problem, with special reference to the north-west of England. By H. W. MILES, D.Sc.

At the Afternoon Session:

II. The Rook in the Rural Economy of the Midlands. By A. ROEBUCK, N.D.A.

III. The Food Habits of the Little Owl. By Miss A. HIBBERT-WARE, M.B.O.U.

Summaries of these papers and of contributions by Mr W. R. S. Ladell and by Mr F. R. Petherbridge to the discussion on the wireworm problem follow.

I. THE WIREWORM PROBLEM

BY H. W. MILES, D.Sc.
Victoria University of Manchester

THE outlines of the wireworm problem are well known to most people interested in entomology and in agriculture and horticulture. Wireworms are the larvae of certain elaterid beetles called click beetles; they live in the soil for 4–7 years and feed on any available crop. The beetles are seldom seen in large numbers, but the wireworm population of the soil may reach hundreds of thousands per acre, and the loss caused by their feeding is probably greater than that caused by any other insect pest. At certain seasons the beetles are attracted to heaps of clover hay and the wireworms assemble in numbers to favoured foods. The usual habitat for the beetles and wireworms is grassland, and the older the grassland the denser the wireworm population. Serious injury by wireworms follows when grassland is broken up for arable cultivation. The insects seem to be most numerous on medium and light soils where intensive agriculture and market gardening are localized.

Various measures for the control of wireworms have been suggested, but the most effective methods involve a heavy expenditure that is only justified under market garden conditions and under glass where the crops have a high value. This Association

has already published accounts of the use of baits in conjunction with the application of calcium cyanide (Miles & Petherbridge), and of the reactions of wireworms to naphthalene (Tattersfield and Gimingham, etc.). The urgent need is for control measures suitable for use under general farming conditions, particularly for the protection of roots, potatoes and cereals. Long range research was begun in April this year at Warburton, Cheshire, and I propose to outline the conditions that make the wireworm problem of special importance in the north-west of England.

The North-West Advisory Province has a rainfall of 35–60 in. a year, which makes it suitable for dairy farming. Certain districts, particularly north Cheshire and south and west Lancashire, have sandy or peaty soils or mixtures of peat and sand, and in these districts intensive agriculture is followed since the proximity of large towns ensures a fairly good return for brassicas, potatoes, beet, carrots and peas. Potatoes and brassicas are also important to the dairy farmer since they are easily produced on his arable land and can be sold when prices are good or consumed at home when prices are poor. A sufficient acreage of grass is maintained by means of temporary leys, an essential feature of Lancashire and Cheshire dairy husbandry, and the usual rotation is oats, followed by potatoes (roots and brassicas), wheat, and seeds for one to three years. Leys are also a feature of the market growing areas; farmyard and stable manure is scarce so fertility is maintained by taking a crop of clover and rye grass and ploughing in the heavy aftermath. The rotation in these districts is oats or wheat followed by clover and rye grass, then potatoes and brassicas; or wheat followed by clover and rye grass, then potatoes and market garden crops, and finally oats. It is apparent, therefore, that throughout the entire region, leys are an essential part of the husbandry and potatoes a constituent of most rotations.

Leys are usually followed by wireworm infestation and where a rotation of four years or less is the practice there is a tendency for the wireworm population to increase steadily. Injury to potatoes receives most attention since attack is concentrated on the tubers, where it is easily seen. In a survey of the potato crop of 1936 it was found that half the farms in Cheshire suffered from wireworm infestation and that loss from wireworm injury to potatoes varied from 2*s*. 6*d*. to 40*s*. per ton. Losses on single farms varied from £24 to £528 and the total loss in the county reached almost £100,000. If to this is added the losses caused by wireworm attack on other crops some idea of the importance of wireworms in the North-West Advisory Province can be formed.

The chief difficulties facing the investigator are that leys are essential in the local systems of husbandry, and that the soils are good potato soils and no other crop can take the place of potatoes economically in the rotation.

Research work has been started along certain lines. Modifications in the rotation are being observed to see if injury to potatoes varies with the position of that crop in relation to the ley. Mechanical processes of cultivation, scuffling, and discing the stubble, which are said by farmers to give some relief from wireworm attack, are being observed in relation to wireworm injury, though it is difficult to see how they affect wireworms other than indirectly. The effect of the potato crop on the subsequent wireworm population is being studied since there is some evidence that numbers of insects are removed with the crop. The effect on the wireworm population of mustard ploughed in green is also under observation since this has been recommended as a treatment for wireworm infested land, but up to the present no direct effect on the insects has been noted. Observations are being made on the movements of wireworms

in the soil, and these indicate two main periods of activity at the surface: late April and early June, and early September to the end of October. Direct control measures with chemicals have not yet been attempted because sufficient biological and ecological data have not yet been collected.

In the discussion following Dr Miles' paper, Mr W. R. S. Ladell said that, at Rothamsted, the problem was being attacked under three main headings: (*a*) Field experiments, (*b*) Chemotropic responses, (*c*) Fumigation experiments in pots.

(*a*) The object has been to find out how far it was possible to test chemical control measures by a field technique similar to that used in fertilizer and varietal trials, and what was the minimum amount of soil sampling that must be done in order to obtain a reasonably accurate estimate of the wireworm population. The distribution of the wireworms in the field was very uneven, and there was no apparent relationship between the numbers present in any one location and any single soil factor such as *p*H.

Three field experiments using old grass land were described. Wireworm populations were ascertained in every case by sampling the soil before and after treatment. At first soil samples $9 \times 9 \times 5$ in. were taken, but with the introduction of the Ladell flotation technique giving a more complete recovery of the wireworms, it was found possible to reduce the size to $6 \times 6 \times 6$ in. Local control was used in the first two experiments and was found effective. The sampling errors were high, varying from 19%, when the numbers of wireworms were large, to 48%, when the numbers were small. In all cases the sampling error accounted for most of the experimental error. Although the errors were high they did not prevent us from detecting real differences between treatments. The first experiment was a 5×5 Latin square, size of plot 1/60th acre, six soil samples per plot. The second experiment was arranged in ten randomized blocks of three plots, size of plot 1/70th acre, four soil samples per plot. The third experiment consisted of eight blocks of six plots, size of plot 1/200th acre, two soil samples per plot.

The mean density of the wireworm population was 65,[1] 335 and 277 per sq. yd. respectively. Good results were obtained by the use of Seekay, Cymag, Chlorpicrin and Crude Naphthalene.

The possibility of using baits instead of direct counts on the soil was being explored. Both in the laboratory and field, cabbage leaf and stalk was found much more attractive than potato. No relationship was found between the number of wireworms obtained in the baits and the actual number in the soil. On plots with the most effective fumigants the baits contained the largest proportion of the real population, indicating a repellent effect of the fumigants into a more attractive environment.

(*b*) Three types of apparatus for finding out the chemotropic responses were described. Of vegetables tried, cabbages were the most attractive, especially cabbage stalk. Under some conditions germinating lettuce was preferred to germinating cabbage. Germinating barley was more attractive than oats or wheat. A few essential oils had been tried and, of these, mint was the best.

(*c*) Much attention has been paid to the design of pots for testing the value of fumigants against wireworms under conditions approximating as nearly as possible to those existing in the fields. Some progress has been made. Certain proprietary mixtures widely recommended were found to be useless. The best results were obtained with carbon disulphide, crude naphthalene and a sodium cyanide mixture, respectively.

[1] Without earlier instars.

Mr F. R. Petherbridge said that, at Cambridge, the work on wireworm has been chiefly in connexion with the sugar-beet crop. Attempts have been made to grow sugar-beet in fields with a high wireworm population. Experiments were designed to find out the value of extra seed rolling, drilling manure beneath the seeds, and drilling wheat between the rows.

In manurial experiments on barley carried out in 1934 by J. A. McMillan & F. Hanley, mixed fertilizers drilled with the seed gave a very much higher yield than did the same fertilizers broadcast in a field where a large number of wireworms were present. At four other centres where few wireworms were present and where these two methods of applying the manure were used, the crop differences were very much less.

In 1935 experiments drilling wheat between the rows of beet gave the largest increase in plant population.

In 1936 in a fen field near Southery (Norfolk) the beet crop was saved by drilling wheat between the rows and the plant population was further increased by extra seeding. The neighbouring plots where wheat was not sown between the rows had to be redrilled.

The following table shows the plant population per acre:

Wireworm experiments, 1937. *Beet population. After singling*, 29 *June*

	Row	Plants per row	Plants per acre
	1......	967	22,531
	..*W*		
	2......	1032	
	..*W*		
Heavy seeding 20 lb. per acre	3......	1027	24,325
	..*W*		
	4......	1044	
	..*W*		
	5......	1073	
	..*W*		
	6......	873	
	..*W*		
Normal seeding 15 lb. per acre	7......	789	18,230
	..*W*		
	8......	686	
	..*W*		
	9......	630	14,679

W = Wheat between the rows.

Where 20 lb. of beet seed per acre was sown and wheat was sown between the rows at the rate of about 40 lb. per acre, the plant population after singling was almost perfect.

It is interesting to note that the outside rows of sugar-beet with wheat on one side only were very much better than the controls, and contained nearly as many plants as the rows with wheat on both sides.

II. THE ROOK IN THE RURAL ECONOMY OF THE MIDLANDS

By A. Roebuck, N.D.A.

Midland Agricultural College

The rook (*Corvus f. frugilegus*) is virtually part of the English country-side. Its large size, its characteristic cawings and its habit of appearing in flocks all the year round compel attention. It is essentially a bird of agricultural land. When feeding it almost invariably frequents farm lands at all times of the year and, as its diet is mixed, its economic position has been disputed from time immemorial.

Some years ago it was decided to attempt a survey of the rookeries in the Midlands, in order to find the number and distribution of the birds. It was decided also to study the kind of food eaten and the total weight consumed annually. Further problems studied have been the possible influence of migration, the competition of other species and the factors which operate as natural controls on the species.

The district surveyed has an area of 5305 sq. miles and comprises the counties of Nottinghamshire, Leicestershire, Rutland, Derbyshire and Lincolnshire. It includes a sea coast of about 100 miles on the East and on the West there are the mountains of Derbyshire rising to 2000 ft. The annual rainfall varies from under 25 in. for most of Lincolnshire to over 50 in. over a large part of Derbyshire.

For the purpose of the census the rookeries were located and the nests counted. By doubling the number of nests the number of nesting birds is obtained. Non-breeding immature birds are not included. The number of these birds varies in different rookeries.

The first survey was made in 1928–30 and this was repeated 4 years later.

The following are the principal data obtained from the first survey:

	Area (in sq. miles)	Number of rookeries	Nests	Birds
Nottinghamshire	843	182	6,501	13,002
Leicestershire	800	230	9,381	18,762
Rutland	152	49	2,340	4,680
Derbyshire	1009	240	10,620	21,240
Lincolnshire:				
Lindsey	1357	442	22,447	44,894
Kesteven	726	160	8,432	16,864
Holland	418	118	4,412	8,824
	5305	1421	64,133	128,266

Average size of a rookery 45·1 nests. 1 rookery to 3·7 sq. miles. 1 bird to 27·4 acres.

The rooks are by no means evenly distributed over these counties. Certain features of their distribution deserve special mention.

In Nottinghamshire they mostly keep to the low ground along the river valleys. There is a large area in the centre, roughly 200 sq. miles in extent, which has no rookeries. Jackdaws are the prevalent birds of the crow family in this area

A large portion of the eastern half of Leicestershire is grassland. On this part of the county the rooks are most abundant. In the grazing district there is one bird to 14·4 acres whereas there is only one bird to 31·3 acres in the rest of the county.

Derbyshire has about 260 sq. miles of land above 1000 ft. high. On this there are thirty rookeries of an average size of 55·5 nests and containing 3324 birds. This represents one bird to 50 acres. More than half of this area is treeless grouse moors with no rooks. On the remainder, therefore, the grazing land on the carboniferous limestone, rooks are as abundant as in the rest of the county, namely one bird to 21 acres. In contrast to the mountains of Derbyshire, Lincolnshire has a large area of fen where the tree tops are higher above the ground than the ground is above sea-level. There is one bird to 30 acres of land. The chalk wolds is the area richest in rooks, there being one bird to 10·5 acres. Although this is arable land there is an abundance of snails as a food reserve on the short leys, etc.

The second survey showed little substantial change in numbers or distribution as indicated in the following table:

	2nd survey		Lost.	Gained.
	Rookeries	Birds	Sites abandoned	New sites
Nottinghamshire	180	12,150	29	27
Leicestershire	235	18,300	31	36
Rutland	46	4,136	8	5
Derbyshire	274	21,774	26	60
Lincolnshire:				
Lindsey	477	44,606	67	102
Kesteven	172	17,018	24	36
Holland	136	9,024	16	34
	1520	127,008	201	300

Their numbers had remained constant, but for various reasons 14% of the nesting sites had changed. These were mostly small rookeries and only about 4% of the birds were involved. This should not be considered a change in 4 years but it is more nearly an annual change. In the intervening years many sites are known to have been occupied and abandoned which do not appear in either survey.

By the autumn the rookeries are deserted and any particular flock may join with a number of other flocks in the district and adjourn to some large wood for roosting at night. There are many exceptions. In some rookeries the birds will roost in trees alongside their nests all through the winter. The large roosts to which united flocks go during the winter are termed rook roosts, major rookeries or winter rookeries.

A survey of these roosts was made and the following numbers located: In Nottinghamshire 8, Leicestershire 13, Rutland 13, Derbyshire 34, Lindsey 58, Kesteven 23 and Holland 4, or 153 altogether.

As a rule the flocks leave the roosts each morning and return to their old feeding grounds for the day. During spells of bad weather, especially if foggy, they are often unable to get far from the roosts.

In order to obtain data on the food consumed by rooks birds were caught in traps and fed experimentally. These were mostly fully fledged young birds. The daily volume limit appears to be 140 c.c. whereas the daily weight has varied from 56 to 127·6 g. In estimations of stomach contents by volume, therefore, 51,100 c.c. must be considered per year, and by weight 56 lb. per bird per year.

The quantity of food consumed by rooks in this area per year thus approximates to:

Nottinghamshire	406·3 tons
Leicestershire	586·3 ,,
Rutland	146·3 ,,
Derbyshire	663·7 ,,
Lincolnshire:	
Lindsey	1402·9 ,,
Kesteven	527·0 ,,
Holland	275·7 ,,
	4008·2 tons

This food is not consumed evenly throughout the year. The surveys have dealt primarily with the nesting birds, that is when the population is at its lowest, but it is obvious that the population varies at different times of the year. One possible effect might have been migration. Numbers of rooks reach the Lincolnshire coast in the autumn but, contrary to what one might have expected, they move north or north-westwards. They appear to pass beyond the county. So far as the Midlands are concerned the writer can find no evidence that migration in any way affects the problem of the rook. With the advent of the nesting season our native population increases. It is impossible to do more than make a rough estimate of this increase in large rookeries. In some small rookeries observed, the population has been trebled by June, each pair having raised four young ones. On the other hand some rookeries fail to rear any at all. On the average the population begins to increase in March, reaches its highest in May, then there is a fall, at first rapid, then slower, until the normal population is again reached in the early autumn. During the months of rearing their young, examination of shot rooks has shown that the proportion of animal food in the diet is highest. It is during these months also that most of the bulk of the food is consumed. Allowing 5% for the non-breeding birds the total food consumed in the Midlands annually is about 4300 tons. Of this quantity 42% is consumed in April, May and June, and 66% is consumed in the period March to August during which time the young are fed by the parents.

The preference for the grazing lands of Leicestershire has been stressed. In addition almost all the rookeries in the area surveyed are alongside a grass paddock. The close proximity of a field of grass seems essential for their welfare. As animal food is necessary during the nesting season it is interesting to consider the distribution of such food on arable and grass land. A rook is not a scratching bird but has to use its beak to extract the smaller animals from the ground. It can only penetrate to a depth of 1¼ in. Published data show that grassland is much richer in insect and other invertebrate life than arable land, especially at or near the surface of the ground. Moreover, on grass, on an average, 62% of this population can be reached by rooks, whereas only 27% can be reached on arable land. It will be seen, therefore, that grass is much more dependable for insect food than arable. It is also easily seen why rooks much prefer to follow a plough on arable land and why they rarely attempt to follow a seed drill.

The difficulties of interpreting data on the analysis of stomach contents are almost insuperable. Assuming a rook ate only one kind of food all the year round, it could consume

585,000 grains of wheat,
or 1,277,500 wireworms,
or 204,400 leatherjackets,
or 81,500 slugs and snails, etc.

The difficulty is that it eats some of each as well as many other things. Moreover, it readily adapts itself to almost any kind of food in an emergency. In the Midlands undoubtedly in the aggregate it confers inestimable benefits to farmers. Average conditions, however, do not exist anywhere. The work done during these surveys indicates that the question of possible damage done is purely local—in the immediate vicinity of the rookery.

III. THE FOOD HABITS OF THE LITTLE OWL (*CARINE NOCTUA* VIDALII)

BY MISS A. HIBBERT-WARE, M.B.O.U.

THE conclusions as to the food habits of this bird are based on the results of the investigation into the nature of the food of the little owl, which was organized by the British Trust for Ornithology from February 1936 to July 1937. The investigation covered a wide field of observation. Over twenty regular observers sent food material to the writer, for analysis, at intervals, daily, twice weekly, weekly or fortnightly. Many others contributed by means of occasional consignments of food material and by first-hand evidence of their observations. In this way thirty-four counties and eighty-one districts were represented.

There were four sources from which evidence as to the nature of the food might be drawn:

(1) The faeces of the bird. The results from these are negligible since nothing but the merest traces of solid matter, e.g. a few rodent hairs, appeared in the faeces.

(2) The food castings or pellets, evacuated from the gizzard by way of the beak. Of these 2460 were analysed in one year, from February 1936. These contained not only the hard indigestible parts of the food, such as bones, fur, feathers and chitinous fragments but also, quite frequently, undigested soft matter such as portions of earthworms and entire insect larvae.

(3) The nest and so-called "larders". Of these seventy-eight were examined; the holes in almost every case were cleared to the base and the contents sent to the analyst. The "larders" contained chiefly the remains of prey taken to these holes for carving purposes before supplying it to the nestlings, e.g. wings of birds and unused portions of mammals. The nests contained superficially other parts of the same animals, e.g. tail quills, legs and contour feathers, portions of the pelts of mammals, etc. The large amount of debris in the nest (sometimes weighing several pounds) consisted chiefly of crushed pellets evacuated by the nestlings and sitting bird. Insect remains were a dominant feature of this debris.

(4) Gizzards of dead little owls. The contents of these sometimes gave valuable evidence of the nature of the last meal taken by the bird. Frequently however the gizzard was found to be empty or with mere traces of mammal hairs, etc., left over from the last pellet evacuation. This was the case with twenty-five of the fifty-one gizzards examined.

The food material obtained from the above three sources show conclusively that the little owl has a habitual diet, alike at all seasons, at all stages of growth and in all parts of the British Isles. This normal diet consists of invertebrates and small mammals. During the nesting season, from May to July, larger mammals and birds

are added in large enough numbers to be included as part of the regular diet, though not to the exclusion of the other items.

A marked feeding habit of the little owl is that it is a ground-feeder to a large extent. The invertebrates found in greatest abundance in the food remains are millipedes, woodlice, earthworms and particular kinds of insects, e.g. earwigs, carabid beetles, dung beetles, weevils and elaterid beetles. Most of these either have no wings or else seldom use them except under special conditions and the fact that their remains are, almost without exception, embedded in a matrix of soil, dung or moss, etc., shows that they were picked up from the ground. Cockchafers are extremely abundant in nest debris and field observers have watched little owls pick them up as they emerged from the puparia in the soil. The great predominance of eggs in the pellets containing *Tipula* (daddy-longlegs) makes it probable that the insects were taken in the act of egg-laying in the soil. In these pellets, obtained from eleven counties in large numbers, two only contained crushed *Tipula* remains without or with very few eggs, the rest were composed of eggs in a matrix of crushed *Tipula*. The field mice, voles, shrews, which form a considerable part of the diet throughout the year and the rats, rabbits (young), house-sparrows, starlings, blackbirds and thrushes which are added during the nesting season are either ground fauna or such as constantly frequent the ground. The normal food of the little owl consists therefore of common ground fauna. There is a very great numerical drop between the animals already named and those on the long list of animals taken occasionally or rarely, but these too are to a large extent procured from the ground.

An examination of the short list of animals which form the habitual diet shows that the little owl must be mainly crepuscular and nocturnal in its feeding habits. The invertebrates and mammals are almost entirely those that are active by night. The four birds commonly used as food from May to July are easily procured at evening and dawn. This inference that the little owl is not much of a day-feeder is also borne out by the following facts:

(1) The field observers have not been able to detect the bird in the act of serious hunting till evening. In two instances they have watched it picking up small objects by daylight, but every observer except one has named from 6.30 p.m. as the hour when hunting began. This observer noticed that near the end of the nesting season, the pair under his observation took to day-hunting. This may frequently happen but it has not been proved to be a habit.

(2) The empty condition of twenty-five of the gizzards examined points to the probability that the pellet, representing the food of the bird during 24 hr., is evacuated after the night's feeding before the bird roosts or becomes inert, and that no serious building-up of a fresh pellet usually begins during daytime. Thus, the habit of the little owl appears to be primarily that of a night-feeder. Daylight hunting is sometimes practised but does not constitute a habit.

Summing up, it can be said that the main feeding habits of the little owl have been shown, in the light of the recent investigation to be: (1) that it has a regular diet common to all districts and seasons, (2) that it feeds chiefly on ground fauna, (3) that it is largely crepuscular and nocturnal in its habits.

Certain food habits often ascribed to the little owl have been disproved by the investigation:

(1) It is frequently asserted that insect food is not used during the nesting season.

Not only, however, has the nest debris been found to be literally studded with clytra, heads and legs of beetles, but the gizzards of the few nestlings sent for examination all contained insect remains. One nestling of about 10 days contained in its gizzard a large fragment of a dor beetle and of a cockchafer. The nest tree can often be detected by means of the accumulation of insect fragments at its base.

(2) It is stated by others that the young are fed on delicate food obtained by raiding the nests of other birds. This investigation has shown that nest-raiding is a rare occurrence, and is not a habit of the little owl. The food of nestling little owls consists partly of larger prey than is used during the rest of the year, namely rats, young rabbits, starlings and blackbirds. Even the beetles used at this season are large species, chiefly cockchafers and dor beetles. There is no predilection shown for delicate food. The aim of the parents is quantity, not quality.

(3) It is further asserted that the young are fed largely on carrion beetles, chiefly burying beetles. It is true that the little owl picks up these beetles occasionally. Seventy-five burying beetles were found in seventy-eight nests and holes and in 2460 food castings. None were found in gizzards. It seems likely that burying beetles are occasionally attracted to the refuse dump in a "larder", also that the little owl turns over carrion lying about, as it does dung and so finds the beetles. But the paucity of the records shows that it is only occasionally that the little owl comes across carrion beetles during its hunting activities. Burying beetles, like stagbeetles, water beetles and many others must rank as occasional, not habitual food of the little owl.

REVIEWS

A Textbook of Plant Virus Diseases. By KENNETH M. SMITH. Pp. x + 615 with a frontispiece and 101 illustrations. London: J. and A. Churchill, Ltd. 1937. 21*s*.

The study of plant virus diseases has shown an almost phenomenal development, since although plant viruses have been recognized since 1892, our knowledge is to all intents and purposes a post-war growth. It has been not only a rapid but, also, an interesting and somewhat untidy development. There has, for example, been no generally agreed opinion as to a technique for the examination of viruses, there has been wide divergence of view as to their classification, and there has been considerable controversy as to their nature. In consequence the literature which, in various languages is scattered in all sorts of journals, is more than usually full of speculative statements, observations, and experimental results the soundness of which is very difficult to estimate. For some little time the need has been urgent for a systematic treatment of the subject to-date, partly in order that both virus workers and general plant pathologists might get their bearings and, partly, that such a treatise should be compiled whilst this was still possible and before the rapidly accumulating mass of data became overwhelming. Further, with Stanley's discovery of the crystalline nature of, at all events, certain viruses it is possible that, in the next few years, the whole study of viruses and virus diseases may be revolutionized. At the close of a scientific period and before attention becomes diverted along the new avenues of exploration which open out, it is desirable that the results of investigations be surveyed critically and co-ordinated by one who has played an active role in the development of the subject. No one is more fitted to this task than Dr Kenneth M. Smith and, in its successful achievement, he has earned the gratitude of all interested in plant disease.

In writing a textbook of a subject of which no previous textbook exists the author must formulate some scheme of ordering and classifying the data. From time to time various methods for the classification of viruses and virus diseases have been proposed, all of them subject to criticism. The scheme adopted in the present volume is a compromise based on the method suggested by Johnson and it will undoubtedly receive criticism, but it has pragmatic justification in that it works. The author groups together all those viruses which are chiefly associated with a particular host plant. These basic viruses are then attached to the generic name of the host and numbered 1, 2, 3, etc., the strains of each virus being lettered alphabetically A, B, C, etc. Thus the numerous viruses chiefly associated with tobacco are grouped as *Nicotiana Viruses* 1 and 1 A–1 D, 2–12 and 12 A–12 B, and 13–15; those associated with the potato plant as *Solanum Viruses* 1–18. Composite viruses are treated similarly; thus the viruses of Rugose Mosaic of the potato, formerly *Potato Viruses* X and Y become *Solanum Viruses* 1 and 2, and the viruses causing Tomato Streak become *Nicotiana Virus* 1 and *Solanum Virus* 1. The total number of viruses included in the book falls into 52 groups each associated with a host name, thus *Delphinium Viruses* 1–2; *Pæonia Virus* 1; *Anemone Virus* 1; *Brassica Viruses* 1–4; and so on. By rigidly adhering to this scheme Dr Kenneth Smith has succeeded in resolving much of the chaos.

Further, throughout his book, the author follows a standard plan of treatment. The virus is first dealt with, its properties, mode of transmission, etc.; and then the diseases it causes, arranged according to the plant families, are described. The viruses as a whole are placed in the order of their plant hosts, Hutchinson's scheme of classification being followed. Thus under *Beta Virus* 1. Boncquet and Hartung, are first given a list of synonyms and the virus itself is then described under the headings:

resistance to various chemicals, alcohol and acetone; miscellaneous reagents; thermal death-point; effect of pH; dilution end-point; resistance to ageing; desiccation; filterability; attenuation and restoration of virulence; transmission. There follows an account of the differential hosts. The diseases caused by *Beta Virus* 1 in plants belonging to ten families are then described, and this section is followed by accounts of the geographical distribution of the virus and of methods for its control. Finally there is a list of the plants forming the host range of the virus. Where data are available for particular viruses or virus diseases notes are also given on electrophoresis, effects of radiation, serological reactions, particle size, crystallization, histopathology, cellular inclusions, carriers, varietal resistance, relation of diseases in various countries, and effect on yield. The balance of the author's consideration varies, naturally, with the state of knowledge concerning particular viruses or diseases. Thus nothing is known of the properties of *Lycopersicum Virus* 5 or *Lilium Virus* 1 whereas those of *Nicotiana Virus* 1 or *Lycopersicum Virus* 3 have received considerable study: with many viruses little is known of the host range whereas, with others such as *Callistephus Virus* 1 or *Cucumis Virus* 1, extensive lists of host plants can be given. The general recognition of these gaps in our knowledge, which become very evident owing to the author's mode of presentation, should stimulate considerable further research.

Throughout all this portion of his book the author's treatment of the subject is characterized by skilful selection of material and precision of statement and, within these seven chapters, he has succeeded in condensing an enormous amount of information and in keeping his discussion remarkably up-to-date. The work is a textbook and not an encyclopaedia of plant virus diseases and, although the vast majority of such diseases receive either description or mention, a few, such as virus diseases of the cactus *Epiphyllum truncatum*, of *Ipomoea batatas*, of *Dolichos biflorus* etc., are omitted whilst, of certain viruses, the author selects the more important diseases pointing out that, owing to their wide host range, it is not practicable to describe every disease produced by them.

Following the systematic consideration of the viruses and virus diseases is a lengthy chapter devoted to the insects, etc., concerned in their transmission. This, as one would expect from the foremost authority on the subject, is extraordinarily well done. A detailed description of each insect usually illustrated by a text-figure is given, together with an account of its life-history and habits, so far as these are known, ecology, food plants, viruses transmitted, and geographical distribution.

Ch. IX contains brief notes on a number of plant diseases which are suspected to be of virus causation but which require further study before they can be placed definitely in this category. Probably many readers of the book will wish to make extensive additions to this chapter but the author has been wise to err on the side of caution.

In the main part of the book the author begins with the viruses and then proceeds to the diseases caused by them: in the field one reverses the process, beginning with the plant showing symptoms of disease and working back to the virus. This practical difficulty is overcome by an appendix, in the first column of which the author lists the host plants alphabetically under their scientific names; in a second column the symptoms of specific diseases are described; and in a third column the names of the viruses causing the particular diseases are given, along with page references to their descriptions in the text. This appendix, which runs to 38 pages and gives the symptoms of 330 virus diseases occurring on 175 different host plants, is an extremely useful compilation.

Following two pages of addenda describing two viruses inadvertently omitted from the work, the book closes with a general index, an index of viruses arranged alphabetically by host names, and an index of authors. The general index does not include the names of those plant species which are merely listed in the text as forming the host range of any particular virus, but only those on which a disease is actually described. A full host index would have been a useful addition to the book.

Nicotiana Viruses 1–12 B occupy Ch. IV, whilst, for some unstated reason, *Nicotiana Viruses* 13–15 are carried over to Ch. V, which also contains *Lycopersicum*

Viruses 1–6, *Hyoscyamus Virus* 1 and *Datura Virus* 1. With the exception of Ch. IV, the bibliography of which is combined with that of Ch. V there is an excellent bibliography terminating each chapter. It is to be regretted, however, that the author has not adopted consistently in his citations the journal abbreviations as given in the World List of Scientific Periodicals.

In addition to the entomological figures of Ch. VIII, the book contains a coloured plate and 77 mostly full-page illustrations each with several photographs, delineating the symptoms of the virus diseases of the several hosts. The need, recognized almost from the beginning of virus study, of accurate photographic portrayal of host symptoms has enabled the author to make his book a very rogues' gallery of viruses and these illustrations, many of which appeared originally in the *Annals*, are a valuable feature of the book. The present volume in no sense supersedes the author's *Recent Advances in the Study of Plant Viruses* published in 1933. It is true that Chs. XII–XIV of the previous work are covered in a more up-to-date way by the specific accounts in the present book but, otherwise, the volumes are complementary and both are necessary to students of the subject.

By his experimental researches on viruses and virus diseases Dr Kenneth M. Smith had already made for himself an international reputation and this will be enhanced by his present volume. This work is not only an invaluable source book of information but the first real systematization and clarification of the science, and it will become the indispensable "Handbuch" of everyone interested in virus diseases.

WILLIAM B. BRIERLEY.

Economic Botany: A Textbook of Useful Plants and Plant Products. By A. F. HILL. Pp. x+592 with 225 text-figures. New York and London: McGraw-Hill Publishing Co. Ltd. 1937. 24*s*.

This is an excellent book. In an introductory chapter the importance and nature of plant products are discussed. The following eight chapters are devoted to industrial plants and plant products and deal respectively with fibres and fibre plants; forest products, wood and cork; forest resources; tanning and dye materials; rubber and other latex products; gums and resins; essential oils; fatty oils and waxes; and sugars, starches, and cellulose products. Two chapters on drug plants and drugs follow, dealing severally with medicinal plants and with fumitories and masticatories. The following seven chapters are devoted to food plants and deal, respectively, with the history and nature of food plants; the major cereals; the minor cereals and small grains; legumes and nuts; vegetables; fruits of temperate regions; and tropical fruits. The remaining two chapters concern food adjuncts, and deal with spices and other flavouring materials and with beverage plants and beverages. An appendix contains a systematic list, with both scientific and common names, of nearly 1000 species discussed in the text. There is a bibliography of 160 references arranged under five subject headings, and the book closes with a good index.

A book covering so wide a field must be selective. It includes the most important plants of America and of other parts of the world in so far as they enter into international commerce, but the author has not attempted to give the detailed morphology of the various species discussed or to consider too fully their agricultural and commercial aspects. Nevertheless, it contains a wealth of information on the botany, history and distribution of the plants, their industrial, medicinal and alimentary uses, the preparation and use of the plant products, and the economic importance of the plants and their products. Often, just sufficient information is given to make one wish to know a great deal more, and one turns to the bibliography, only to be slightly disappointed. The bibliography cites too many textbooks, and old editions and other works which have been superseded by more recent publications. Other than English and American references are almost entirely excluded, and no one of the great French books on economic botany is cited.

The book is up-to-date, well and interestingly written, and most pleasantly free from inaccuracies and misprints. Here and there one reads sentences which are a little surprising; e.g. "Plants have been and still are responsible for many of the social ills that beset mankind", "Perhaps the chief social problem for which plants are responsible is the narcotic drug habit and the illicit trade that has grown up around it", "the African natives...cannot be taught proper methods of tapping", "in the past the (Chinese) nation as a whole has shown in its mental and physical characteristics the effects of the opium habit", and so forth.

The author states in his preface: "Even though the value of including a considerable amount of economic material in a beginning course in botany may be recognized, the limitations of time or various curriculum requirements usually render such a procedure impracticable. It should be possible, however, to offer at least a half-year devoted to economic plants as a supplement to the usual first year's work." Coming from a member of the botanical staff at Harvard this is interesting and, if his point of view is at all widely held by American University teachers of botany, they must differ considerably from most of their English confrères. A difficulty hitherto, suggested as partly responsible for the active or passive opposition to the inclusion of economic botany in the ordinary botanical curriculum, has been the absence of easily available English textbooks on the subject. With the publication of the present work, the books by Stanford (*Economic Plants*, 1934; *General and Economic Botany*, 1937), the excellent little book by Good (*Plants and Human Economics*, 1933) and older books by Robbins and Ramaley, Barrett, Clute, Kraemer, and others, this difficulty can no longer be made an excuse for a situation which is due primarily to sheer conservatism and mental inertia.

Dr Hill has written an exceedingly useful book which should be in every botanical library and in constant demand by teachers and students alike.

WILLIAM B. BRIERLEY.

Practical Plant Breeding. By W. J. C. LAWRENCE. Pp. 155 with 34 illustrations. London: Allen and Unwin Ltd. 1937. 5*s*. 6*d*.

This little book by Mr Lawrence, Curator of the John Innes Horticultural Institution, is the best introduction I know to plant genetics. The author introduces his subject by describing the structure of flowers and the processes of pollination and fertilization, and follows this by a clear and practical account of the technique of breeding. Two chapters deal with the laws and mechanism of inheritance, and three remaining chapters with sterility, and the methods and results of plant improvement. The book opens with an appreciative foreword by Sir Daniel Hall, and closes with a short list of books for further study and an index.

The book is very clearly written and the author has been unusually successful in condensing and translating into simple language a mass of technical research in this difficult field. Indeed, considering its small size, the book covers all the main issues of the subject in an extraordinarily efficient way. It is packed with well chosen examples and is well illustrated.

In numerous ways Mr Lawrence has produced just the book that many of us have been hoping for; a small cheap book, understandable, accurate and up-to-date, giving just the right amount of necessary detail and yet maintaining a broad and suggestive outlook. It is ideal for amateur gardeners and nurserymen or for students of horticulture.

The only pity is that the book is purely horticultural since, in many institutions, students of agricultural botany and of horticulture combine for a short course in plant genetics. If, in a second edition, Mr Lawrence could include examples from agricultural crop plants so that the book would also appeal to students of agricultural botany, its size would not be greatly increased but its value would be doubled.

WILLIAM B. BRIERLEY.

British Stem- and Leaf-Fungi (*Coeolomycetes*). Vol. II. *Sphaeropsidales and Melanconiales*. By W. B. Grove. Pp. ix+407, with 102 text-figures. Cambridge: University Press. 1937. 21*s*.

Vol. I of Mr Grove's book which dealt with the *Sphaeropsidales—Hyalosporae*, *Hyalodidymae*, *Hyalophragmiae* and *Scolecosporae*, received notice in the *Annals*, 23, 3, 1936. The second volume, which has now been published, completes the *Sphaeropsidales—Phaeosporae*, *Phaeodidymae*, *Phaeophragmiae*, *Dictyosporae*, the *Nectrioideae*, *Excipulaceae* and *Leptostromataceae*, and includes the *Melanconiales*. The general viewpoints and methods of treatment exemplified in Vol. I are continued throughout Vol. II. There is the same meticulous accuracy of nomenclature and diagnostic detail and the same pungency in Mr Grove's comments, many species erected particularly by Cooke or Massee, being criticized in no uncertain language.

Concluding the systematic treatment proper are three pages of addenda to Vol. I, and four pages of diagnoses of new genera and species. There follow an epilogue and a poem by Mr Grove both of which are entirely out of place in a book of this character. The book closes with indexes of Ascomycetes, of host plants, and of binomial names, an additional note on *Diplodia*, and a list of authorities with their correct abbreviations.

In his epilogue Mr Grove mentions that he was born in the year 1848, and the production of such a work as this at the age of 89 is an extraordinary achievement. Mycologists will not only felicitate him upon the attainment of so ripe an age but offer to him their gratitude for the splendid results of his labours.

WILLIAM B. BRIERLEY.

Plant Ecology. By Hilda Drabble. Pp. 142, with 12 Plates. London: Edward Arnold and Co. 1937. 7*s*. 6*d*.

A good elementary introduction to ecology. The first eight chapters deal with the mode of life of plants as individuals, and with ecological terms and concepts, and the remaining eighteen chapters with plant communities in relation to their habitats and to each other. There are a brief epilogue containing hints on study, a short bibliography of books and papers for further study, forty-five text-questions arranged by chapters, an index to plant names, and a general index. The book contains an Errata slip of six items which needs to be greatly extended. The photographic illustrations are excellent.

WILLIAM B. BRIERLEY.

The Properties and Functions of Membranes, Natural and Artificial. Pp. 911–1151. London: Gurney and Jackson. 1937. 12*s*. 6*d*.

This volume contains the papers and discussions of the Fifth Colloid Meeting of the Faraday Society held in April 1937. It is reprinted from the *Transactions of the Faraday Society* with the original pagination. The subject was discussed under the following heads: Part I. Natural Cell Membranes: (*a*) General—structure, permeability, membrane potential, anaesthesia; (*b*) Special—red cells, fish gills and egg membranes, plant cells, bioelectric phenomena; (*c*) narcosis. Part II. Artificial Membranes. There are fifteen papers and discussions in Part I and nine in Part II.

Many of the papers, although fascinating from a more theoretical standpoint, are of perhaps rather remote interest to applied biologists. Some, however, have direct bearing upon research in various fields of applied biology, and the attention of Virus workers particularly may be drawn to the paper by W. J. Elford on "Principles Governing the Preparation of Membranes having Graded Porosities. The Properties of 'Gradocol' Membranes as Ultra-filters."

The papers are interesting but very condensed statements of the position to-date of the various problems discussed, and the volume forms a splendid cross-section of this extraordinarily wide and difficult field.

WILLIAM B. BRIERLEY.

Perspectives in Biochemistry. Edited by JOSEPH NEEDHAM and DAVID E. GREEN. Pp. viii+361, 5 plates and a Frontispiece. Cambridge: University Press. 1937. 15*s*.

No person stands higher in the esteem of the scientific world than Sir Frederick Gowland Hopkins, and few have had so great an influence on their science either through their own researches or through the men and women who have gathered inspiration at their hands. It was therefore a singularly happy thought of his past and present students to celebrate his seventy-fifth birthday by writing and presenting to him this book of essays. It is a fine tribute paid to a great man.

Reflecting the wide interests of the founder and head of the Cambridge Biochemical Laboratory the essays touch on many aspects of the science of life with all its great bearing on human welfare. Physiology and zoology, embryology and genetics, medicine, bacteriology, and nutrition all pay homage, and essays on "The Biochemistry of the Individual" or "The Meaningless of the Terms Life and Living" join hands with essays on "The Economy of the Bacterial Cell", "The Chemical Regulation of Insect Growth", "The Biochemistry of Flower Colour Variation", "Biochemistry and the Pathogenic Viruses", and numerous other problems.

The aim of the writers has been to indicate the most promising lines of advance in the various fields which they survey, and while maintaining a due standard of criticism, to speculate a little on the likely paths of future thought and discovery. Each essay deals concisely with a particular topic of which its author has special knowledge and concludes with a bibliography. The essays are well written and interesting to read and, where all are so good, it is impossible to select individual essays for mention. One may, however, be forgiven for quoting a short passage from Prof. Marrack's essay on "The Social Implications of Biochemistry" since this shows well the potentialities of Sir Frederick's life-work. "We are no longer dealing with vague principles but with chemical compounds, whose physiological activity may be correlated with physical and chemical properties. As the sense of power over nature which this knowledge gives spreads out from the specialist to the general public, men will gather confidence to abandon traditional beliefs and inhibitions and to shape a society in which all the possible resources of science and production are used for the good of all men."

WILLIAM B. BRIERLEY.

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SOME RECENT DEVELOPMENTS IN VIRUS RESEARCH

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ADDRESS OF THE RETIRING PRESIDENT OF THE ASSOCIATION OF APPLIED BIOLOGISTS, DELIVERED TO THE ANNUAL GENERAL MEETING ON FRIDAY, 11 FEBRUARY 1938

I PROPOSE to-day to discuss only two aspects of virus disease. One is the thoroughly practical problem of control, the other the highly abstract problem of the nature of a virus. To both problems noteworthy contributions have been made in the last two or three years, and it is my purpose to outline for you this progress. I shall not offer any original contributions of my own, but I do not think I need make any apology on that account. It is almost impossible for anyone not a specialist to keep up to date with the output of virus literature, and even for the specialist it is not easy: the mass is so overwhelming. When I was confined to bed at the beginning of the year, reprints kept piling up on my desk at the rate of more than one a day; and it is a new and interesting test of the state of one's health when the thought of this accumulation ceases to be a depressant and becomes a stimulant. The switch-over may be taken as the beginning of convalescence, and I suppose one might take as a sign of exuberant health the day when one was really thrilled by the description of a new virus disease in the potato plant. If that is so I am still below par—and have been for some years.

To such an audience as I have to-day I need hardly insist on the economic importance of virus disease at the present time. You must all of you have had personal experience of the damage it causes. We are passing at present through an outbreak of foot and mouth disease, and the daily press has been filled with estimates of the financial loss this outbreak has caused and is still causing to the country. But every year virus disease in plants produces losses on a similar scale, not only in this country but all over the world. It attracts little popular attention because it is so normal. It is rather like the death roll on the roads: we are too accustomed to it. Attempts are made to reduce it but, as in the other case, with only indifferent success. And yet the urgency of the question needs no emphasis.

It is not easy to get any precise quantitative estimate of the loss in any one crop due to virus disease; and quite impossible to make a worth-while estimate of the aggregate loss in this country or the world as a whole. We know that, in sugar cane, mosaic disease some years ago was so serious as to threaten the destruction of the industry in Louisiana and the West Indies. In sugar beet the incidence of the curly-top disease varies greatly from year to year, largely according to the magnitude of the insect infestation. In a bad year the average yield per acre may fall from the normal of 11–15 tons to 1 or 1½ tons; but, taking an average over a number of years, bad and less bad, the loss on the 763,000 acres in the western states of America is reckoned at about two million tons a year. In this country, in the potato crop virus costs about two million pounds a year. And this is one of the few cases where the estimate may be accepted as reasonably accurate. Every year about 500,000 acres are planted with potatoes in England and Wales. Of these 500,000 acres about 120,000 are planted with fresh seed tubers. The remaining 380,000 acres are planted with tubers from locally grown plants. We know that these local tubers contain a certain amount of virus, which increases progressively the longer the local stock has been in use. On the average replacing the local tubers by fresh seed raises the yield by 1 ton per acre. At the low valuation of £5 per ton that represents an annual loss in the 380,000 acres not sown with fresh seed of about two million pounds in the potato crop of England and Wales due to virus disease.

I quote these figures merely as examples to show the magnitude of the problem. If we could add to the potato losses of this country those in the glasshouse industries, in strawberries, raspberries and other small fruits, in hops, bulbs and in the flower trades, the figures would be very large indeed, even for England and Wales, and almost incredible if we could assess the loss throughout the world.

The whole problem of control falls into two main subdivisions: (1) the sowing of clean material; (2) the keeping the crop clean during the period of growth.

In the case of crops which are grown from true seed, the first is in the majority of instances fairly easy, because it is only in a few cases that virus is carried through the true seed. It happens in some of the Leguminosae and in one or two other cases. Whether it occurs in tomatoes and cucumbers, as has been maintained in this country, is still a matter of dispute. It is not really a very easy thing to settle, because it is notoriously difficult to prove a negative. There is a lot of evidence against it and some for it. Myself, I should not be inclined to be dogmatic either

way, because I am not sure that the disputing parties are always talking about the same things. It is at least possible that the evidence against is based on work with different viruses from that in favour. But, whatever the truth may eventually turn out to be in that particular question, it is not a very difficult matter to ensure that in crops grown from true seed the seed sown shall be free from disease.

But in the case of crops vegetatively propagated—that is to say, from tubers, bulbs, sets as in sugar cane, or suckers as in raspberries, and so on—the problem of starting with clean material is very much more difficult. One cannot without elaborate testing be sure that the plant from which one takes the tubers or bulbs or cuttings is in fact free from infection, even although it shows no external evidence of disease. It may have been infected only a short time before the propagating material was taken and contain the disease although not showing it. Or it may be in a carrying condition, either as a permanent carrier which never shows any symptoms at any time, or as a temporary carrier which shows symptoms for only a short time and then loses all visible signs.

This difficulty has given rise to elaborate methods of investigation of the plants which are to be used as the parents of the new crop. In this country you are familiar with the system of inspection of the growing potato crop, and the issue of certificates that the crops inspected contain only a specified small percentage of virus disease. In America there is an extensive system of tuber indexing, which is in effect a preliminary testing for the presence of virus before the issue of the tubers for planting.

These methods work out on the whole with a fair amount of success. There are, of course, cases where the inspection and certificates are misleading. This is not always due to fault on the part of the inspectors. The inspection is a visual one, and the potato crop in particular is liable to a masking of symptoms, which is dependent on the weather. After a bright sunny spell symptoms are much less evident than after a cloudy, wet, muggy period, and a crop may seem fairly free from disease at the times of inspection, when it is actually widely infected. But on the whole it works fairly satisfactorily, and there is a considerable probability that certificated seed is comparatively free from virus.

So that both with crops that are grown from true seed and crops that are vegetatively reproduced there is a reasonable chance that one is starting with comparatively clean material.

But it is a very different matter once the seed is planted and the crop is growing. It is then exposed to infection over a period of weeks or

months, and it is impossible to prevent this exposure by any practicable means known at the present time. The sources from which the infection may come are manifold. There is the source within the crop itself—in potatoes, for example, the small percentage of diseased plants among the parents supplying the tubers used as seed. Or it may be residual plants left in the ground from a previous crop. This is a frequent source of contamination. In the Sudan, for example, the incidence of leaf-curl in cotton came down astonishingly when an efficient means was devised of getting rid of the roots of the previous crop. In the tobacco industry the influence of residues and the effect of soil composition on the duration of infectivity of such residues have been demonstrated by James Johnson. In this country we have the ground keepers left after the harvest of the potato crop. These supply foci of infection within the crop itself. In addition there are the external sources: infected plants within carrying range, either plants of the same species or biennial or perennial weeds, which have been shown many times to be a reservoir for carrying over the infection through the winter. There is no reason to believe that insects themselves can to any significant extent carry over the disease from one season to the next. Those which are responsible for the bulk of virus spread do not commonly survive a winter in the adult stage, and there is only one authenticated case where infection has been shown to be transmitted through the egg. There is no difficulty in finding explanations, valid at least in theory, for the entry of a variable number of infections into the growing crop.

Every point of entry is a source of spread. There is no doubt that in most cases the agency of this spread is insect carriage. There are, of course, other mechanisms, which are sometimes operative. In diseases transmissible by juice the rubbing of an infected plant against a healthy one, especially under the action of wind, may transmit infection. You will have noticed a recent letter in *Nature* in which Prof. Murphy demonstrated that this occurs in potato with the virus X; and the same thing has been noted in tobacco. But this can be responsible for only a small amount of spread. It is too slow a process to be a very serious danger, and the scattered incidence of the disease over a growing crop shows that there are other more effective means. There is no satisfactory evidence of transmission through the air; or—except in one disease of wheat not known in this country—through the soil, apart from the presence of infective residues. There are some crops where mechanical transmission does play a very important part, namely, those in which the individual plants are handled by the cultivators. The stripping of the leaves in the

tobacco crop and the stopping out in tomatoes are examples. In such cases disease is spread broadcast on the hands of the operatives, and the juice of an infected plant carried to healthy plants widely through the crop. But these are special cases. In most instances the diseases are spread throughout a growing crop by the agency of insects.

This is very clearly seen in the curly-top disease of sugar beet in the United States. The virus has a very extensive host range, and its hosts include many of the plants on which the vector, *Eutettix tenellus*, a jassid, lives. In the spring the vector develops to an enormous extent on the weeds of the foothills or uncultivated plains, and when this vegetation begins to dry up as the season advances, the insects migrate to the cultivated areas and attack the sugar beet, returning in the autumn to the areas from which they came, ready to descend once more in the following season. The damage to the crop is closely correlated with the magnitude of the insect invasion. In years when the insects come in relatively small numbers, the damage from curly top is small; but in a bad insect year the virus invasion is enormous, and, as we have seen, the yield may fall from 11–15 tons an acre which is the normal down to 1 or 1½ tons.

What determines the size of the insect invasion is still obscure—at least in detail. Climatic factors are obviously largely responsible, and it seems clear that unusually cold winters and early springs reduce the numbers. We have still a lot to learn about the variation in insect numbers from year to year, and the death of Maldwyn Davis, a member of our Association, has been a serious loss to virus entomology, because that was the type of problem which he was attacking with marked success.

There are, of course, cases in which the vector is unknown. The transmission of tobacco mosaic, for example, is one of the most puzzling problems to the plant pathologist at the present time. Here we have one of the most infectious diseases known, transmissible by the tiniest possible drop of infected juice, and yet of the many insects that feed upon the tobacco plant none is known to carry the disease, although one might have expected that of all diseases this would have been the most readily carried. In our own country virus *X* is one of the two most important sources of disease in the potato crop; but its vector—whose existence no one, I imagine, really doubts—is still unknown.

The practical problem of control, then, reduces to the questions—how are we to limit the invasion of the growing crop, and how are we to limit the spread from those points where entry has been gained?

The direct and finally most satisfactory method would be to interrupt the transmission by control of the insect. But as yet no practicable method of doing so has been devised. In limited areas and in special crops of high financial value, it may be possible by repeated spraying to reduce the incidence, as was shown by Mrs Watson for the small *Hyoscyamus* crop grown for medicinal purposes. But such a method is not practicable for large crops, such as potato, because of the expense. One might conceivably introduce into the ground some cheap chemical which would make the plants distasteful to the insect, but this has never so far as I know been suggested or tried. As yet no success has been obtained in interference with the insect.

Until recently the only general method that has had any real success has been the development of immune varieties. This has been conspicuously successful in the case of the sugar cane. Races have been developed—originally by the Dutch in Java—which give a high yield of sugar and are highly resistant to the mosaic which is the principal danger to the crop; and these strains are now grown throughout the world whereever the sugar cane is grown. There are, of course, recurrent difficulties. A strain which is immune to the virus of one locality may not be immune to the virus of another locality. You will remember the similar case of Biffen's rust-immune wheats. And even in the one locality a new strain of virus may appear, to which immunity has not been obtained, as has indeed happened in Louisiana. When this occurs, a new genetical complex must be arranged to meet the new conditions; and this takes time and it is not every industry that can bear the expense. But, undoubtedly, immune varieties have largely solved the sugar-cane problem. In other crops the success has not been so great. In the Sudan a strain of cotton has been obtained which is highly resistant to leaf curl and gives a fairly satisfactory product. In sugar beet, as the result of long and very costly investigations, some strains have been evolved which give a fair yield and, while not immune, have enough measure of resistance to make them commercially useful; and no doubt these strains will be further perfected. But apart from these crops the use of immune varieties has not been particularly successful. In this country, for example, we have no resistant varieties in our main virus-affected crops, and progress along this line has been very small.

In the last few years, however, a new method has been devised. It is still in the experimental stage, and it is not yet possible to estimate how successful it will be; but it is very promising. It has been found that the presence of a virus systemically distributed throughout a plant protects

that plant against subsequent invasion by certain other viruses. The fact was first described by Thung in Java, who was working with the diseases of tobacco; but his paper escaped the attention of any but the Dutch workers, and its possible practical significance was not at first appreciated. It was discovered independently by Salaman in Cambridge, who was working with potato viruses, and shortly afterwards was described by Kunkel in America, and almost simultaneously by Caldwell in this country, both of whom worked with diseases affecting tobacco and tomato. All these workers arrived apparently quite independently at the same results, but so far as I know it was Salaman who first realized the practical significance. Since these first reports a considerable amount of work has been devoted to the phenomenon, and a number of further facts established. It was soon shown that the protection conferred was in some sort specific. It did not hold against all viruses—that, of course, was evident from what we already knew about mixed infections—but only against viruses related to the original protection-conferring virus. Infection with potato virus *X* or *Y*, for example, gave no protection against subsequent infection with tobacco mosaic; nor preliminary infection with tobacco mosaic protection against *X* or *Y*. But infection with ordinary tobacco mosaic protected against later infection with aucuba or yellow mosaic, a more virulent strain of tobacco mosaic; and infection with a weak strain of *X* protected against later infection with a virulent strain. There is no need for the first strain to be a weak one: one virulent strain will protect against another related virulent strain. It is, however, not always easy to demonstrate the protection in the case of virulent strains, because the symptoms of the one strain mask or obscure the symptoms of the other.

The nature of this protection is still obscure. It would seem that the presence of the first strain does not prevent the entry or dissemination of the second, but it prevents it from exerting its normal destructive action. The first strain, wherever it establishes itself, blocks, as it were, the second strain, occupying the available ground, so that when the second strain arrives it finds a squatter already there and no opportunity for establishing itself, except in occasional areas that have escaped the first. A certain amount of time is necessary for the first strain to occupy the ground, and this period varies with the respective aggressiveness of the two strains: it may require a few days or several weeks. If the second strain is inoculated at the same time as the first, or before the necessary interval has elapsed, there is either no protection or a mixed infection results, in which the more virulent strain gradually dominates the other

and may oust it entirely in the later growth of the plant. But if the necessary interval has elapsed the second strain makes no headway, and apparently—though I am not sure of this—eventually dies out.

Thung has examined a considerable number of tobacco diseases from this point of view and has grouped them according to their penetrative powers and their dominance with respect to one another. But I need not go into details here, especially as he has not always examined the relationships of the strains to one another. The salient fact with which I am here concerned is that it is possible in some cases and perhaps in most to confer protection against a severe disease by a previous infection with another allied strain which may be of quite a mild type.

Now from the commercial point of view the grower does not care whether his crop is diseased or not, so long as the disease does not affect adversely either the quality or the quantity of the crop. And this is a not infrequent happening. There are some strains of tobacco mosaic known, for example, which produce merely a quite trivial and sometimes indistinguishable discoloration of the leaves. In potatoes some of our standard varieties are 100 % infected with a mild form of mosaic that reduces neither the number nor the quality of the tubers. In America practically every potato plant contains a latent virus which, in suitable varieties, has no perceptible effects.

Here then is a possibility of a practical commercial protection against severe disease. If one can infect in advance one's crop with a mild form of the common serious disease, one has protected it against that serious disease, and it does not matter how much it may be exposed to it during the period of growth. The crop is diseased, but its disease does not affect its commercial value.

This possibility is in course of trial at the present time. In Holland, Prof. Quanjer is carrying out extensive investigations on potatoes and with results that are, so far, most encouraging. In Cambridge, Salaman is also investigating the possibilities, and also with promising results. There is necessary, of course, a lot of preliminary research to discover suitable weak strains which shall give satisfactory protection without themselves causing significant damage; and he has now got strains both of *X* and *Y*, which are effective in laboratory conditions and are being tried out on the field scale. Only experience will show whether the protection will persist through several generations, whether it will be possible to combine in the one plant several protective infections against several of the severe types, whether the principle is applicable to all sorts of plants, and so on. But one may say that a new principle has been

discovered which may solve in a practical manner some part at least of the problem of control.

The method you will notice is essentially a vaccination. Now it is obviously impracticable to inoculate every plant in a standing crop of many acres. One can infect a certain number of foci and leave it to the natural means to spread the infection throughout the crop, hoping that this early infection will get to work before the severe type comes along. That perhaps is what one may have to do in the case of crops grown from true seed—at least those crops where virus is not transmitted through the true seed. But in the case of vegetatively reproduced crops the protection may be made almost universal. One can ensure that the parents from which one's propagating material is taken are already protectively infected, and it may be possible to build up races and stocks of plants immune to the graver types of disease, all of them infected but with infections that are not of practical significance to the grower. And as these races get spread throughout the country, the destructive types of disease may be gradually eliminated altogether.

The endeavour to obtain control, therefore, has taken a definite and a hopeful step forward in the last year or two.

I turn now to a very different subject, namely, the nature of a virus. As you know, this has been a subject of debate since the first discovery of the existence of viruses. In almost the first detailed account of the virus of tobacco mosaic Beijerinck described it as a *contagium fluidum vivum*, and since then there has been a continual interchange of amenities sometimes metaphysical, sometimes almost mystical, between the living and the non-living schools. On the one hand it was maintained that they were simply small organisms, no doubt with special properties because of their demonstrably small size and the consequent great increase of surface relatively to their bulk, but still essentially organisms analogous to small bacteria. On the other it was maintained that they could not be organisms in any ordinary usage of the term, that they were essentially non-living, and that, although they could not really be like the enzymes with which we were already familiar, they were autocatalytic complexes more akin to enzymes than to any autonomous creature.

The difference of opinion persisted because there was nothing known which was decisive one way or the other; and the view adopted probably depended on the way the disputant had been educated. If he was a chemist he naturally inclined to the non-organismal view; if a biologist to the small bacterium theory, and both were happy and argumentative. On the whole, I think the balance of opinion was, for many years at least,

definitely in favour of the organismal view. Since viruses could not be grown on cell-free media, they were held to be obligate parasites, and the fact that they required their food to be prepared for them in some way by the host was correlated with the smallness of their size. Apart from that condition of their existence, they were organisms as much as any other organism. They varied in size in a descending scale from comparatively large bodies to bodies which could be rendered visible only by photographing them with short wave-length light; and they continued to look like organisms in the photographs as each successive refinement of the photographer's art, each progressive reduction of the wave-length used, enabled one to penetrate further and further down the scale.

But, as research progressed, the organismal theory became gradually less convincing. It was shown by filtration through collodion membranes that some at least of the viruses were smaller in size than single molecules of known proteins such as the haemocyanins, and Laidlaw found a sewage organism which grew well in cell free media and yet was of similar size to many accepted viruses. It was difficult to believe that a thing the size of a single molecule could possess all the properties of life; and it began to be realized that no one could say what they meant by an organism, nor define precisely what is the difference between a thing that is living and one that is non-living.

Then came Stanley with his claim that he had isolated a crystalline protein with all the properties of tobacco mosaic virus. Now there was nothing new to the plant pathologist in the idea that virus is associated with crystals. Iwanowski pointed out thirty years ago that tobacco mosaic disease was characterized by the appearance in the cells of flat plate-like crystals not found in normal cells and exhibiting cross striation under the action of acid. Very much later, about 1931, when attempts were being made to obtain virus in a purified state, free from the non-specific ingredients of the plant, the idea was mooted that the virus might itself be crystalline. True crystals which had infective properties were in fact actually obtained, and the conception of a crystalline virus became familiar. But these crystals were soon shown to be really crystals of the materials used in the process of purification, and their infectivity was due to virus entangled as they formed. It was not till Stanley's paper came out that the possibility of a genuine crystalline virus had to be seriously entertained.

The assertion that the virus was crystalline was responsible for much of the attention at first given to the new claim, for those of us who had been brought up in the older order of ideas attached a kind of sanctity to

the word "crystal". It was a sort of guarantee of purity, and Stanley himself cited as evidence of the purity of his protein that it could be recrystallized as many as 15 times without change of properties. As a matter of fact, repeated recrystallization, at least in the case of large molecule proteins, gives no such guarantee of purity, and in the particular case of the crystalline material of Stanley, obtained by precipitation by ammonium sulphate, it has been shown by Bawden that it is demonstrably impure and retains its impurity, however many times it is reprecipitated. Moreover, it has been shown by Bernal that these so-called crystals of tobacco mosaic are not really crystals in the full sense of the word. They have some of the orderly molecular arrangement found in crystals, but so have many other things not usually described as crystalline, such as muscle fibres or hair structures. They have only a two-dimensional regularity instead of a three-dimensional, and they are better described as fibres or paracrystals. Actually it is a matter of secondary importance whether the protein as found appears to be fully crystalline or not. The observed form may be determined by the conditions under which the isolation is effected, and if these conditions were suitably modified, full three-dimensional crystals might result instead of the incomplete two-dimensional form. Even as it is, the protein can under certain conditions be obtained as long mesomorphic fibrils visible to the naked eye. But undoubtedly the crystalline property attributed to tobacco mosaic caught the attention of many who might otherwise have been slow to appreciate the significance of the new step.

At the same time as Stanley was working on tobacco mosaic, Bawden & Pirie in Cambridge had been isolating virus proteins from potato mosaic. The potato mosaic viruses do not give, or at least have not yet given, the needle fibres, but when Bawden came to Rothamsted and applied to tobacco mosaic the methods used with potato, he obtained the same needle crystals as Stanley's and most of his results were confirmed. Bawden & Pirie, however, carried the work a good deal further than Stanley and most of what I am about to say is based on their results.

There is no doubt that the material isolated from the juice of infected plants has most of the properties of tobacco mosaic. It is infective in concentrations of the order of one-millionth of a gram per c.c., increases very rapidly in the infected plant, and is transmissible in series from plant to plant indefinitely. The disease produced is identical in every respect with the disease of the plants from which the material was obtained in the first instance. With one important exception, with which I will deal in a moment or two, it has all the properties of the virus found

in naturally infected plants, such as resistance to chemicals, ageing, heat, enzymes, etc.

There is also no doubt that it is a protein. It gives all the usual reactions, and its analysis conforms to that usual in known proteins. Bawden & Pirie consider that it is a nucleoprotein but the American workers have not yet accepted this view, at least not in print. The discrepancy has a certain importance because of the implications and associations which a nucleo-protein nature suggests; but that is a technical matter which will no doubt soon be cleared up. Its composition is constant whatever the source of the material analysed and whatever the concentration of the substance it contains. It can be obtained from every host plant that the virus can actively infect. It is, therefore, not a substance peculiar to the tobacco plant or the tobacco-virus complex, but appears wherever the virus is able to multiply, even in hosts in no way related taxonomically to the tobacco plant, such as phlox or spinach.

It has not been found in any normal plant, even in very small amounts, and the methods of extraction are delicate enough to reveal it, even when it constitutes only a millionth part of the plant tissue examined. In the infected plant, on the other hand, it occurs in surprisingly large quantities. The juice of an infected plant contains from five to ten times as much soluble protein as that of a normal plant, and about 80 % of this soluble protein consists of the abnormal substance. From one to two grammes can be obtained from a litre of sap, the amount varying with the condition of the infected plants and the duration of their infection. Where all this excess protein comes from is unknown. It is conceivable that it is a modification of the protein already existing in the normal plant. Possibly the existing protein, perhaps the non-soluble portion, is converted into the new soluble form, and the cells, requiring the normal protein for their own purposes (and one must remember that the diseased plant, though damaged, is still a functioning organism, growing to a large size) are stimulated to replace the converted material by more of the original, which is in its turn converted, and so we get an accumulation of the new form, giving a total content of soluble protein much greater than is normally found. This theory would imply that the creative force, the synthetic or constructive power which converts the normal material into the abnormal, resides in or is a property of the abnormal substance itself. But there are other obvious possibilities. It may be, for example, that the *cell* produces the abnormal protein under the stimulus of the abnormality, a theory which evades the necessity of giving reproductive powers to the protein itself. The fact is that we have as yet no evidence

at all as to the mechanism or the source of this huge development of a foreign substance.

From solutions of the protein can readily be obtained needle-shaped bodies resembling crystals which are visible under the microscope. They average in length about two to three hundredths of a millimetre. They can easily be dissolved and again obtained, and the process can be repeated indefinitely. But as, I have said already, it has been shown by Bawden that these crystals are not necessarily pure, and contain a fraction which is not virus protein and can be removed by digestion with trypsin. This has been beautifully demonstrated, by means of the anaphylactic reaction, by Chesters, who showed the presence of the impurity in the American ammonium sulphate preparation and its absence in the purified material prepared by Bawden.

When a solution of the protein reaches a certain concentration it reveals a new property, becoming birefringent and showing anisotropy of flow. This property indicates that the constituent particles are rod-shaped. When the concentration is high, so that the rods cannot move about freely, but are necessarily arranged in parallel bundles for lack of space—like matches in a match box—the solution is permanently birefringent. When the concentration is of a lower order, the rods are able to move comparatively freely in all directions and there is no birefringence; but on the formation of currents or eddies in the liquid they assume the parallel arrangement in these areas, lying longways in the direction of the flow, with resultant anisotropy of flow. (This has been ingeniously shown by Bawden & Pirie, who put a goldfish in a solution of the protein, and the movements of its tail produced eddies which showed the birefringence.) The property is greatly affected by impurities, notably by breakdown products of the virus protein, and it is not possible at present to estimate the length of the rods with any precision though it seems that this must be at least ten times as great as the width. The width can be determined accurately from X-ray analysis, and is found to be 150 A. The cross-sectional area amounts to 20,000 A.^{2}, and is probably triangular, and these dimensions are constant for all concentrations of the protein.

The molecular weight and the particle size could be derived from the rate at which the particles come down in a centrifugal field, if the particles were spherical. Since they are rod-shaped it is scarcely possible to arrive at a really reliable value for the particle size. The cross-section shows that the molecular weight is large, and if we take the length as ten times the width and the specific gravity as 1·37, the minimum molecular weight

must be of the order of 20 millions. This is enormous. It is larger than the largest haemocyanins, and is approached only by one known substance, a thyroglobulin polymer which is estimated to have a weight of 15 millions. The new protein is at least a very unusual substance, unlike anything found in the sap of normal plants.

There is reason to believe that these rods are not the ultimate constituents, but are aggregates of subunits arranged in linear form. This is indicated by the fact that in the purified state the protein has lost the outstanding character of a virus, namely filterability. Every operation which precipitates the protein such as simple high-speed centrifugalization, precipitation by alcohol, by acid or by ammonium sulphate, entails this loss of filterability. We know that, in untreated sap, the virus can be filtered through membranes of a pore size of 53 mμ., but when it is purified it will no longer pass a membrane with 450 mμ. pores. It looks as if in the juice and, probably, in the living plant the virus exists in a smaller, more discrete form, but in the isolated state it has undergone an aggregation or polymerization—which must be linear, because the width remains the same. The existence of such subunits within the particle, of a length of about 20 A., is shown by the X-rays. There is other evidence that in the crude sap the protein is present in a different molecular arrangement —for instance, the absence of anisotropy in the sap, although it is a solution of 0·2 %, and the smaller infectivity of the isolated virus whose serological titre, nevertheless, remains the same as that of the virus in the sap. Up to the present it has proved impossible to disaggregate the protein again by any method tried.

Proteins of this type have now been isolated by Bawden & Pirie from three strains of tobacco mosaic and two strains of cucumber mosaic having a serological affinity to tobacco mosaic. The diseases are clinically distinct, and the corresponding proteins are also characteristically distinct. The protein varies as the virus varies. From other diseases, such as certain of the potato mosaics, these workers have obtained infective nucleoprotein precipitates which are amorphous and do not give the needle crystals, and which have a different composition on analysis. These proteins are susceptible to trypsin and so cannot be purified by its means, but they can be obtained by high speed centrifuging. Very interesting, too, is their recent work on bushy stunt of the tomato since, from that disease, they have isolated infective proteins which are truly crystalline. They are regular in all their dimensions, and therefore, of course, do not show double refraction; but they are infective in very low concentration, and in this case also there is no reason to doubt that

they are the virus. Other viruses examined in America have yielded similar proteins, so we have good reason to believe that a protein constitution of virus is not a unique phenomenon found only in tobacco mosaic.

There remains now to consider the important question—is this protein actually the real virus? If we could be quite certain that the purified protein is really homogeneous, really pure, the question would not arise. So far as intensive study has shown, virus proteins have been prepared which do seem to be really homogeneous. But it is always open to any one to assert that they are not, and that the virus is really present as an impurity in the protein preparation. Although the protein is present only when the virus is present, and is specific, it may yet be a reaction product produced by the plant in response to the virus, it may be a symptom just like any other symptom, and its infective properties be due to the presence in it of the true virus, from which it has not yet been separated. This assertion is one which cannot be directly disproved. One remembers the presence of heavy water in water thought to be pure and of argon in pure nitrogen. There is always a possibility that some test as yet unthought of, or some increased refinement of the existing tests, may reveal such impurity. The theoretical possibility must always remain, but the mass of evidence against it is now so large that we are entitled to disregard it, and the onus of proving its existence is transferred to those who assert it.

Certainly there can be no large impurity or inhomogeneity. The constancy of the product obtained from the most varied sources is enough to show that much; and also the infectivity which is regularly demonstrable in a concentration of 10^{-8} to 10^{-10}. Neither test is very refined, but nothing has been found that would suggest the presence of an impurity at all. Any procedure that removes protein lowers the infectivity, and the activity declines *pari passu* with degradation of the protein. The temperature or the degree of acidity or alkalinity which destroys the protein also destroys the activity, and it has not been found possible in any way to dissociate the protein from the virus. The hypothetical contaminant must have the same isoelectric point. It must also have the same molecular weight, since in the analytical centrifuge the pure protein gives the sharp sedimenting boundary of a pure molecular species. In short the contaminant must have the same physical properties as the protein and it is gratuitous to postulate the presence of two substances where one is enough to satisfy the data.

It would seem, then, that in the case of tobacco mosaic at least the old dispute between the vitalists and non-vitalists had been resolved in favour of the non-vitalist hypothesis. For, certainly, whatever exactly

the vitalists do mean, they would not consider that a pure chemical substance possesses all the properties they assign to organisms. It has, however, been maintained that all the work I have been describing to you does not exclude the possibility of the virus still being an organism. All that has been shown, says this argument, is that the isolated material has the property of linear aggregation, possibly even of true crystalline aggregation, under certain conditions of stress, such as high centrifugal force or precipitation, and that it gives the chemical reactions of a protein. But it has not been shown that these aggregating units are not themselves organisms. The isolated material, it is granted, is the virus, but why should not the ultimate units still be organisms and function as such in the disaggregated condition which they have when actually in the plant? Here again we come up against the question of homogeneity and, to some extent, against terminology. But there are positive objections to this argument. No known organism consists exclusively of protein. So far as we know at present, every recognized organism contains diffusible constituents, which can be leached out by appropriate means; but the virus proteins contain no diffusible constituents of any kind. Again, in every known organism, water is an integral part of its make-up, bound up with it and united to it in an intimate association. But that is not the case with the virus proteins. It does not unite with water. Water may penetrate between the particles, and does so to an extent which varies with the concentration of the solution. But it is a purely external relationship. The actual particles remain the same, as is shown by X-ray analysis in all concentrations of the material. Further, it has been shown by Bernal, again by the X-ray, that within these actual particles the structure is regular, and these regularities persist at all concentrations. The scale of this regularity is of the order of 20 Angstroms, very small therefore, smaller than any particle hitherto observed that may be claimed to be a living organism, and in character they are intermediate between a denatured and an undenatured protein. If there were any more complex material there, any vital constituent within the particle, it could only be a quite small fraction of the bulk of that particle. There does not seem to be any ground for the view that the constituent particles are organisms in any ordinary meaning of the word.

We are driven, then, to the conclusion that in the case of tobacco mosaic and its allies the actual virus is a protein, a chemical substance. But that is not to say that all viruses are proteins and nothing else. We know already that even within the tobacco mosaic group the infective proteins differ among themselves and have characteristic properties

which distinguish them from one another. We know, too, that while such a virus as the potato virus yields an infective protein, of liquid crystal type, it has not yet given anything but amorphous precipitates on isolation, and on analysis it is different from the tobacco mosaic protein. And, as I have said, Bawden & Pirie have recently isolated from the bushy stunt disease of tomato a protein which is fully crystalline, showing no double refractive flow, and with yet another analytical composition. It does not require much imagination to conceive that there may be a scale of increasing complexity of composition among the viruses, and that as one goes up the scale one may eventually cross the shadowy boundary between what may be called living and what must be called non-living. There may in fact be viruses which are truly organisms. But it does seem to be established that there are some viruses which are not.

AN INVESTIGATION INTO THE "STRIPE" DISEASE OF NARCISSUS

I. THE NATURE AND SIGNIFICANCE OF THE HISTOLOGICAL MODIFICATIONS FOLLOWING INFECTION

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(With Plates VI and VII and 2 Text-figures)

Introduction: Symptoms of the disease

During the past few years it has become increasingly evident that the disease known as "Stripe" is spreading rapidly among commercial stocks of Narcissus in this country. Nevertheless, our knowledge of the disease has hardly advanced since 1932 when Hodson (1932) pointed out that singularly little accurate information existed regarding the trouble. Although there is usually little difficulty in recognizing "Stripe", the symptoms displayed by affected plants vary so widely that some doubt exists as to whether it is a single disease or whether the name, as used at present, is really being applied to several different diseases which have not yet been recognized as separate entities, and which may later be shown to be caused by different pathogens.

The one feature common to all cases so far observed is a discoloration of the foliage and flower stalks due to abnormal distribution of chlorophyll in the tissues. Even this symptom, however, does not present a uniform appearance throughout the group, both the colour and shape of the affected areas varying, though they are usually constant within a variety. Two main types of discoloration have been observed. In one, the colour ranges from a green only slightly yellower than the normal to a bright yellow, while in the other it is a silver grey. The yellow-green type may take the form of longitudinal stripes of varying length and width or of mottling, the affected areas being rounded in outline and frequently covering a large proportion of the leaf surface of the affected plant. The silver grey type is present as longitudinal stripes extending practically the entire length of the leaf. The flower colour is also broken by light streaks or patches in some varieties.

In addition to one or other of these forms of discoloration other symptoms are frequently present. In some varieties the surface of the leaves becomes roughened owing to the formation of longitudinal corrugations of varying length. This condition is seen in an extreme form in the variety Czarina where ridges of tissue stand out prominently from the surface. The roughening usually occurs in discoloured areas, but the surrounding tissue is sometimes quite normal though the ridges themselves are yellow-green in colour. A somewhat similar condition may be found after bulbs have been subjected to hot-water treatment for eelworm, but from our observations it would appear that the ridges of tissue so produced are not discoloured in the manner typical of those associated with "Stripe", and there is, further, a tendency to cork formation which is not apparent in comparable symptoms in the diseased condition. The occurrence of the ridges on the surface of diseased plants is more widespread than might be supposed from superficial observation, for while they are obvious in some varieties, such as Czarina, they are detected only by very close observation in others in which they remain small and inconspicuous.

An entirely different symptom is found among infected plants of certain "Trumpet" varieties, notably King Alfred, the plant being severely distorted and commonly bent through a wide angle. In these cases the discoloured areas are often bright yellow, especially on the inner curve of the bend, the outer curve of which is frequently normal in appearance. Minor distortions of flower stalks and leaves are not uncommon, leaves quite often turning through a right angle in the plane of the surface.

The symptoms described in the preceding paragraphs are those encountered by us during the past growing season, either on the bulb farms visited or in our own experimental gardens and houses. There may well be others which we have not so far observed, for, as we shall show later, the origin of these symptoms is such as to allow of wide variation in the appearance of affected plants.

HISTOLOGICAL MODIFICATIONS CORRESPONDING TO DIFFERENT TYPES OF SYMPTOMS

As a first step towards a better understanding of these apparently dissimilar symptoms and of the disease, an investigation was made into the modifications of internal structure resulting from infection. Work on "Stripe" is severely handicapped at the present time by the lack of any technique for artificial transmission of the disease. McWhorter (1932) claims to have transmitted the apparently similar "Grey disease" in

America, but the inoculation methods tried in this country have not so far proved successful with "Stripe". The scope of this investigation is therefore restricted to an examination of plants showing various types of symptoms collected from bulb fields and our own gardens.

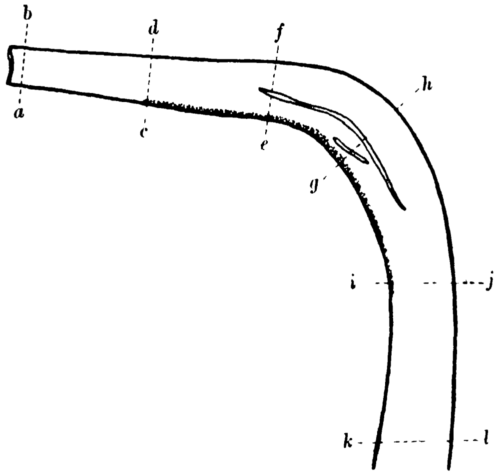
The ridges of tissue produced in the leaves of Czarina were first examined, and by cutting sections of a large number of leaves showing different stages of development the origin and mode of growth were determined. The palisade tissue in healthy leaves is represented by a single layer of elongated cells varying little in length except towards the edges of the leaf where they are more nearly isodiametric. A cross-section of a leaf from a diseased plant shows occasional cells in this layer to be devoid of chlorophyll and slightly larger than the neighbouring cells. The epidermal cells bounding such parts of the palisade are usually larger than normal. This is the first stage in the development of the ridges. The affected palisade cells continue to enlarge especially in the radial direction, where the spongy mesophyll offers less resistance than the palisade tissue which is more closely packed in the tangential plane. At the same time pressure exerted on the epidermis tends to push apart the cells in spite of their increased size. Eventually the enlarged palisade cells divide, a cross-wall being formed in the tangential plane. The products of division lengthen and, by this time, the tips of the cells protrude through a gap in the epidermis where two adjacent cells have been pushed apart. The width of the abnormal tissue as seen in cross-section depends upon how many adjacent palisade cells in the transverse direction are affected; usually two or three enlarge simultaneously at the beginning. In the longitudinal direction rows of cells of considerable length behave similarly, thus forming the ridge instead of a rounded projection. Further division and elongation of the newly formed cells result in the production of a tissue of appreciable size, replacing the palisade and projecting beyond the surface of the leaf. It has been noticed that the form of the cells in these proliferations varies even on the same leaf. In some they are long and narrow while in others they are short and practically isodiametric. This difference depends entirely upon the relation between the rate of elongation and the frequency of division. In some cases division of the palisade cells takes place before any appreciable elongation has occurred (see Pl. VI, fig. 3), and the daughter cells also divide before they have become much larger, thus producing a small-celled tissue as opposed to the large, radially elongated cells resulting when division is delayed until considerable increase in size has taken place. Intermediate conditions between these two extremes may also be seen, but whatever the final

form of the cells the origin of the proliferation can always be traced to the palisade. Pl. VI, figs. 1–3 illustrate the initial changes in the cells of this tissue which lead to the ultimate formation of the ridges in Czarina. The behaviour of the epidermis varies. Occasionally the rate of division and growth of the cells results in an increase in length of the epidermis which keeps pace with the production of tissue from the palisade, and in this case it remains unbroken, bounding the proliferation on the outside. When this happens it is usually found that the cells below are of the small isodiametric type denoting frequent divisions. Where the palisade cells elongate appreciably before division they push apart the epidermal cells at a comparatively early stage.

The origin of the ridges in Czarina having thus been established, a number of other varieties showing similar symptoms were examined. In every case the same developmental stages could be recognized. Among the varieties examined were Sir Watkin, Weardale Perfection, Minister Talma, Emperor and Golden Emperor. Attention was then turned to varieties in which no distinct ridges are apparent but in which the surface of the leaves becomes roughened locally, and it soon became evident that there was no essential difference between the origins of the roughened areas and the ridges in Czarina. The first specimen examined was a plant of Princeps, and the same proliferation of the palisade was observed. Fewer divisions take place however, so that less new tissue is produced. The ridges thus project but little beyond the surface, and as fewer adjacent rows of cells are involved they are also narrower than those found in Czarina. A number of such ridges running parallel and close together make the surface slightly rough to the touch although the corrugation is inconspicuous and not easily recognizable.

In a further investigation plants were selected in which the only noticeable symptom of the disease is discoloration. A good example of this type is afforded by one of the Triandrus hybrids. This variety shows extensive discoloration in the form of yellow-green mottling, but no ridges or roughening of the surface are apparent. We do not propose to discuss here the effect of the disease on chlorophyll or the origin of the different colours observed in affected regions, beyond stating that no chloroplasts have been seen in the cells of mature proliferations or in badly chlorosed areas. The significant fact for present purposes is that palisade cells in these plants show exactly the same tendencies as lead to the production of ridges in the varieties previously examined. Occasional cells are seen to be larger than their neighbours and devoid of chlorophyll. The epidermal cells opposite these sections of the palisade

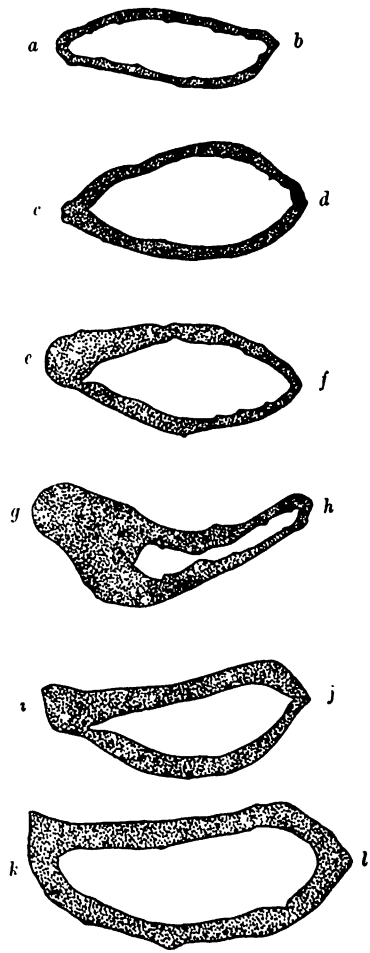
are often enlarged also. Less frequently a few divisions occur in the palisade mesophyll producing small ridges even less conspicuous than those in the roughened areas already described. Were they more closely grouped they might even so be detectable, but the affected rows of cells are rarely close together. Ridges are occasionally found in which division and enlargement have proceeded as far as in those causing roughening of the surface in other varieties, but being narrow and isolated they are not easily observed except by examining a section of the leaf. This is more particularly the case towards the end of the season, when the tendency to proliferation becomes more pronounced. Many plants of different varieties showing yellow-green discoloration were examined and all



Text-fig. 1. Distorted flower stalk in a "striped" specimen of Mon Tresor.

showed the same signs of incipient proliferation. This is true also of those in which the leaves showed silver grey striping, but in this type additional modifications are apparent. Not only is the palisade of the affected region lacking in chlorophyll but the spongy mesophyll is also chlorotic. The palisade cells divide without much enlargement, and there is a rather loose tissue of isodiametric colourless cells below the epidermis. The cells towards the surface of the leaf have thicker walls than those farther in. We have not yet seen any positive indication that the spongy mesophyll, besides having no chlorophyll, takes part in the proliferation, but it is difficult to rule out this possibility. The absence of chlorophyll from this tissue as well as from the palisade no doubt accounts to some extent for the distinctive colour of these areas.

The next plants to be examined were those showing severe distortion. A good example was found in a specimen of the variety Mon Tresor. The flower stalk curved through a complete right angle, the inner edge of the bend showing severe chlorosis and the outer edge being green and apparently normal. This appearance is typical of distortions of the type commonly known as the "King Alfred Stripe". The stalk was cut transversely at several points on the curve and above and below it, and Text-fig. 2 shows the form of the cross-section at the levels indicated by dotted lines in Text-fig. 1. At the level of the first cut (*a–b*) the hollow stalk consists of a ring of tissue of practically the same depth at all parts. The outermost tissue is an epidermis of the normal type with numerous stomata. Immediately below is a single layer of cells forming a continuous ring, well packed with chloroplasts and resembling the palisade layer in the leaves. Inside this the cells form short chains with wide air spaces between, making a tissue similar to the spongy mesophyll. The remaining tissue of the stalk consists of large thin-walled cells containing no chlorophyll. At a rather lower level (*c–d*) there is a slight increase in the depth of the ring of tissue at that part of the circumference corresponding to the inner edge of the curve. This increase is due to the production of a tissue of rather small isodiametric cells containing no chlorophyll and with slightly thickened walls. The epidermis remains intact, the growth keeping pace with the increase in circumference of the stalk. The new tissue lies immediately below it, and when this becomes



Text-fig. 2. Cross-sections of the flower stalk illustrated in Text-fig. 1, at the levels indicated by the dotted lines.

extensive the differentiation between the outermost layer and the remainder of the cortex disappears. The whole area lacks chlorophyll, and there is gradual transition from small cells with thick walls beneath the epidermis to large thinner walled cells towards the centre. The latter show no change from medullary cells occupying a similar position at the higher level. A series of sections cut at different levels and including the region of transition from the normal structure to the abnormal thickened region afforded some indication of the origin of the new tissue. The first sign of any irregularity is the occurrence of cells containing no chlorophyll and slightly larger than the normal in the layer immediately below the epidermis. The elongation, though sufficient to be significant, does not proceed very far. The cells may occasionally double their length but never, so far as we have seen, exceed that. The tissue resembling the spongy mesophyll in leaves is devoid of chlorophyll but does not appear to take part in the proliferation. In the early stages it can be recognized by the characteristic arrangement of the cells, lying between the products of cell division and the medulla.

The next cut (*e–f*) revealed an increase in the extent of proliferation, and the cross-section at the middle of the curve (*g–h*) showed the maximum increase in thickness. Here the small-celled tissue has grown to a thickness many times that of the ring of tissue in the normal parts of the stalk and, furthermore, extends round an appreciable proportion of the circumference. The epidermal cells and those below, besides developing thick walls, show a tendency to form cork so that a tough compact tissue results having little elasticity. Subsequent sections at progressively lower levels show a similar sequence to those above the curve, and eventually the normal condition is again found in the straight part of the stalk. At the middle of the curve the normal structure is completely destroyed in the region where the proliferation occurs and longitudinal growth ceases. In other regions at the same level the structure is perfectly normal and there is abundant chlorophyll in the cortex. Hence with normal growth proceeding except in one sector of the circumference the stalk grows round the inextensible region forming a bend. As the proliferation is only of limited length, normal growth takes place both above and below the affected region. In the course of examining the stalk very much smaller proliferations were encountered having precisely the same mode of origin and development as the large one. These caused no distortion and produced ridges on the surface resembling those found in Czarina. The occurrence of distortion therefore appears to depend entirely upon the extent of proliferation, particularly in the tangential plane.

DISCUSSION

It is evident from the observations recorded above that the disease has three noticeable effects on the internal economy of the plant:

(1) Inhibition or destruction of chlorophyll causing discoloration of affected regions.

(2) A stimulus to cell division in the epidermal and palisade tissues of the leaf and in corresponding tissues of the flower stalk.

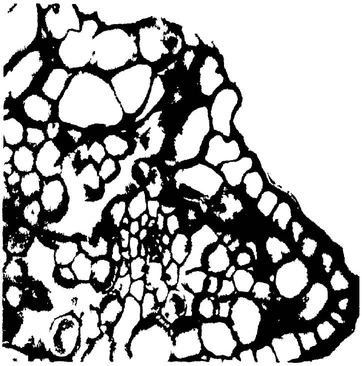
(3) A stimulus to growth of individual cells in the tissues mentioned in (2).

While each of these effects has been observed in all the varieties examined, the relative magnitudes are by no means constant and the visible symptoms vary accordingly. Since the appearance of a diseased plant depends upon a combination of three factors, each of which is capable of wide variation in degree, the number of possibilities is extremely large and is made even greater by the production of secondary effects such as distortion. The development of a particular effect in a plant depends upon the internal and external environments rather than uneven distribution of different casual agents. The influence of the internal environment is shown by the distinctive nature of the symptoms developed in each variety when several are growing together under similar conditions, while the power of the external environment is shown by the modification of the symptoms under changed conditions. Some plants of the variety Princeps growing in our experimental gardens were noted as being badly infected with "Stripe" at the beginning of 1936. The most obvious symptom was a well-marked chlorosis of the leaves, and towards the end of the season there was a slight roughening of the surface. During the summer some of the bulbs were lifted and planted in pots in a heated glasshouse. This season the plants in the house showed only very faint chlorosis but more marked roughening of the surface, while those in the gardens again showed severe discoloration (see Pl. VII, fig. 1). A similar result was obtained with plants of a Triandrus hybrid. A plot of this variety in our gardens showed severe yellow-green mottling on the leaves, and on 27 February two rows of bulbs were planted in pots and transferred to the heated glasshouse. The subsequent growth of the leaves of these plants showed such a diminution in the severity of the chlorosis as to appear practically clean, whereas the fresh growth of the leaves in the gardens showed mottling of the same intensity as before (see Pl. VII, fig. 2). With the advent of warmer weather, however, the same reduction of mottling became evident in these plants also, so that eventually leaves from both sets of plants pre-

sented a similar appearance. There was a severely mottled area at the tip and practically clean growth below. Since the leaves grow from the base the distance the mottling extends from the tip affords an indication of the relative periods of exposure of the plants to cold conditions, and a number of measurements gave a mean distance of 6·4 in. for plants grown throughout in the gardens and 4·5 in. for plants transferred to the glasshouse in February. The discoloration in the mottled areas becomes less marked after a time in the warmer conditions, and some plants which only appeared above the ground after the weather had become warmer appeared almost clean, suggesting that the disease inhibits the formation of chlorophyll rather than destroys it and the inhibition becomes less effective as temperature rises. The tendency to proliferation, on the other hand, becomes stronger under the warmer conditions. We have noticed more pronounced ridges on the leaves in the heated house than on the leaves of plants of the same variety growing outside, and many varieties produce detectable proliferations only in the warmer weather towards the end of the season when the discoloration may practically disappear.

The causal agent of "Stripe" is still obscure, but from our observations there would seem to be at least three components each having different optimal conditions for activity. Although all three are present in a diseased plant, the different behaviour of individual cells gives the impression that they can exist separately and are not uniformly distributed throughout the tissues affected by the disease (see Pl. VI, fig. 3). The symptom picture, the reaction to environment, and the observations recorded here are all suggestive of a virus complex, and although the disease has not yet been transmitted artificially we believe it will eventually be shown that such a complex is the cause of "Stripe". In this connexion it is of interest that during the examination of sections from diseased plants we have frequently noticed large bodies in the cells, lying close to the nucleus and resembling the X-bodies produced by some virus diseases. These bodies have not so far been seen in healthy plant material. Owing to the simple nature of such bodies and the lack of any characteristic feature by which they can be identified it cannot be stated that those observed during this investigation are necessarily the result of virus activity, but we have seen them only in diseased material although we have examined a large number of sections from healthy plants. They can be seen both in stained and unstained preparations but are most apparent after the fixing and staining techniques used by Sheffield (1931) for the examination of X-bodies. Pl. VII, figs. 3, 4 are photographs from sections treated in this way and show the bodies in question in the epidermal cells of infected leaves.

Fig 1



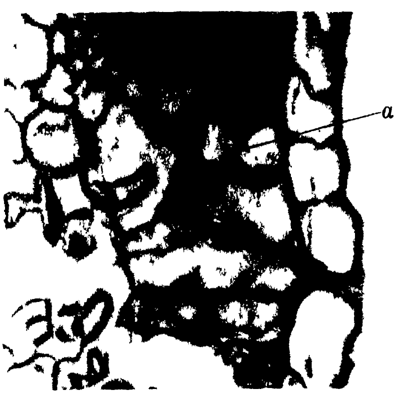


Fig. 3.

Fig. 1

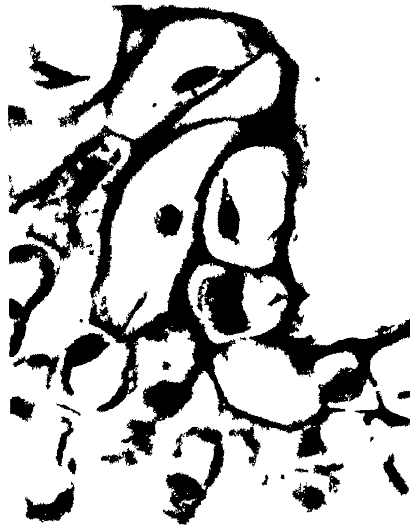


Fig. 3

Fig. 2

Fig. 4

CALDWELL AND JAMES—AN INVESTIGATION INTO THE STRIPE DISEASE OF NARCISSUS (pp. 244–253)

SUMMARY

The wide variation in appearance of infected plants gives rise to the question whether the name "Stripe" is being applied to one or more diseases. The histological bases of the various types of symptoms are described, and it is shown that they are all produced by the same three factors acting with varying degrees of relative intensity.

It is suggested that the disease is caused by a virus complex having at least three components. Inclusion bodies in the cells of diseased plants are described which resemble the *X*-bodies associated with some virus diseases.

This investigation is being carried out under the auspices of the Agricultural Research Council.

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EXPLANATION OF PLATES VI AND VII

PLATE VI

Fig. 1. An early stage in the development of a ridge on the surface of a leaf from an infected plant of Czarina. Palisade and epidermal cells have enlarged but no division has yet occurred. The section is taken near the edge of the leaf where the palisade cells are more nearly isodiametric than they are in the central region. Note the inclusion body lying alongside the nucleus in one of the enlarged palisade cells.

Fig. 2. A rather later stage than that shown in Fig. 1. An enlarged palisade cell has divided forming the two cells on the left of the proliferation. A gap has been made in the epidermis through which elongated palisade cells are protruding.

Fig. 3. Transverse section of a leaf of Czarina showing elongation of palisade cells without division and (at "*a*") a cell which has divided after comparatively little enlargement.

PLATE VII

Fig. 1. Leaves of Princeps, (*a*) from the glasshouse, (*b*) from the gardens.

Fig. 2. Leaves of a Triandrus hybrid, (*a*) from the gardens, (*b*) from the glasshouse.

Figs. 3, 4. Sections of leaves from diseased plants showing the inclusion bodies in epidermal and palisade cells.

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THE ANTITHETIC VIRUS THEORY OF TULIP-BREAKING[1]

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(With Plates VIII and IX)

THE word "antithetic" is here applied to two naturally associated viruses to connote differences in symptom expression which relate to some inherent antagonism between the two viruses. The antithetic virus theory of tulip-breaking states that typical breaks result from the presence within the plant of two viruses which, on a physiological basis, are not only distinct but are also antithetic. One of these is called Tulip Virus No. 1, or "colour-removing"; the other is called Tulip Virus No. II, or "colour-adding". The theory, originally formulated in 1931, required a four-year period for proof and demonstration. Its confirmation involved isolation of the viruses, determination of symptoms induced by each, and finally, the production with measured virus mixtures of typical broken tulips comparable to commercial Rembrandts. This paper is a record of these steps.

TERMINOLOGY

It is proposed that the term "breaking" be accepted as the general name not only of the effects of tulip viruses on tulip flowers, but also as the general designation of the effects of viruses on other flowers where similar pigmentation disturbances are brought about. For example, the flower colour change in *Lathyrus odoratus* infected with mosaic is associated with virus activity. Breaking in tulips, according to popular usage, refers to removal, addition, or rearrangement of anthocyanin pigments in various areas of the epidermis of the flower. If the usage of the term is restricted to these specific occurrences, it should not be confused with "flecking", which results from thinning of the ground

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tissues into hyaline areas, a condition well shown in flowers of mosaic diseased narcissus.

In the present paper an attempt has been made to avoid additional terminology in describing broken-tulip patterns. The literature, both popular and technical, is laden with terms such as "clotted", "flamed", "feathered", "flag", "splashed", "full", "self", "dark", "white break", "red break", "Bizarres", and "Bybloemen". The antithetic virus theory excludes the need of most of these except for what might be termed cataloguing. The terms "full breaks" and "self-breaks" as used by workers at the John Innes Horticultural Institution seem especially apt, and are here given preference. These are interpreted as follows:

Self-break. The condition in which darkening of the flower is prominent. This may result from a local increase in the density of epidermal pigments or an addition of epidermal pigments. The ground colour may not be exposed. The pattern may be clotted, feathered, or evenly diffused.

Full break. The condition in which epidermal pigment is removed; the exposed ground colour then becomes the dominant colour of the flower.

Typical break or average break. The condition characteristic of commercial Rembrandts where the balance between epidermal pigments and exposed ground colour (white or yellow) produces a "flashy" or "flag" break. The usual colour of the flower is intensified in some areas, unchanged in other areas, and is removed in still others.

In the following tables and discussions, Virus I refers to colour-removing virus; Virus II to colour-adding virus. The symptoms or types of break induced are represented as follows: Symptom-type-1 refers to full break (as described above), Symptom-type-2 refers to average break, and Symptom-type-3 refers to self-break. It will be explained that the Symptom-type induced is a function both of the viruses present and of the tulip variety concerned.

HISTORY

It has been suggested that there are two or three kinds of tulip-breaking, each effected by a different virus. In 1929, Brierley,[1] while working with bulb viruses at the Oregon station, recorded "yellow mosaic" plants among Farncombe Sanders Rembrandts and made specific

[1] Brierley, P. Unpublished notes dealing chiefly with insect transmission studies, a part of which is summarized in the *U.S. Department of Agriculture Yearbook* for 1928.

inoculations with them. Before this, McKay & Warner (1933) had collected a broken purple tulip which evinced an exceptionally dark break and which was designated as "Marie Stewart mosaic", from an obsolete or local varietal name. He concluded: "In the experimental work completed at the time this review of the literature was undertaken, there was evidence of more than one, possibly three, types of mosaic disease in tulips." Hughes (1934) gives a favourable consideration to the antithetic virus theory, but slightly over-emphasizes the etiological significance of added colour patterns.

The antithetic virus explanation of tulip-breaking was proposed by McWhorter (1932) as a *theory* dependent on general observation of the following facts: first, the extreme variation shown in a population of approximately 2000 Farncombe Sanders Rembrandts used in natural spread tests in 1930–1; second, the record on inoculations made by Brierley in 1929, and repeated by McWhorter in 1930 and 1931, indicated that the type of break induced is directly related to the type of inoculum used; third, the fact that outlying shoots of large, typically infected Farncombe Sanders clumps often bear distinctly darker or lighter flowers than are borne by the inner portions of the clump. This third observation suggested that the self and full breaks and their accompanying leaf patterns might be accounted for by two agents (viruses) which move at unequal rates through clumps and hence induce different symptom expressions in different shoots of the same clump; and that the "kinds of mosaic" used in previous inoculations were merely natural segregates from average virus combinations brought about by the breaking up of clumps in the usual cultural practices.

To prove this hypothesis it was necessary to start with a large, typical full-break clump in which natural virus segregation was evidenced, encourage natural segregation by selective breaking up of the clump, and use the secondary clumps as sources of inocula. If the hypothesis was valid these secondary clumps should yield different viruses. After the viruses were thus isolated, it was necessary to show how they could be recombined for controlled synthesis of tulip breaks. This work is presented in the following tabulations and discussions in support of the antithetic virus theory. A similar method for the separation of plant viruses was first employed by McKinney (1931).

All of the flower records were made on field-grown plants, because under greenhouse conditions colour-removing symptoms are retarded, inhibition of chlorophyll is modified, and the symptoms as a whole are masked.

INOCULATION TECHNIQUE

The hypodermic needle method of inoculation is very successful with tulip viruses. A standard inoculum consists of juice expressed from tulip stems and leaves, filtered through cloth and diluted 1 to 10 or 1 to 20 with sterile distilled water. Inoculations are made into stems while the plants are in bloom or shortly thereafter by means of a 20-unit Luer syringe and small (No. 27) needle. The success of the method depends on the use of a small needle. Four or five units of inoculum per stem are sufficient to insure a high percentage of transfer. Sister-bulb checks are used in all important tests, so that each inoculated plant is checked by a non-inoculated sister plant derived from the same bulb clump. The details of this method, which is comparable to the tuber-unit system widely used in potato work, are given in a paper on iris mosaic by Brierley & McWhorter (1936). All of these records refer to field plantings left in place for 2 years. The first year the pairs, consisting of bulbs planted in sister-bulb sets of eight, sixteen, or twenty, are rogued to obviously healthy matched pairs; then one plant of each set is inoculated. The tabulations of inoculations here presented list the number inoculated and the number which survived the following year. The effectiveness of an inoculation is indicated by the number of clumps in which symptoms developed divided by the number of inoculated clumps which survived. The few survivals recorded in some cases resulted from the depredations of gophers (*Thomomys bulbiverus*) and moles (*Scapanus townsendi*).

ISOLATION OF VIRUS I OR COLOUR-REMOVING VIRUS

It is well known that some commercial Rembrandts tend to produce flowers of reasonably constant pattern; others tend to show great individual variation. Noticeable variations in the chlorophyll distribution in the leaves may accompany the flower differences. Farncombe Sanders Rembrandt is an example of a named variety which varies greatly in appearance and development. Fortunately there was available for study at the Oregon station in 1930 a stock of over 2000 of these Rembrandts, among which two chief groups or segregations were clearly discernible. One group was composed of weak plants with strongly striped, sometimes yellow striped, leaves and much whitened flower; the other was composed of plants with mottled leaves and average full-break flowers. The striped condition, favoured as it had been in this case by the deliberate saving of weak plants, represented a natural segregation of the Virus I, which, as will be shown later, is dominant. Selected plants

among these weaklings were the sources of inocula for further Virus I segregation. Continued selection of these weak Rembrandts will obtain an almost pure Virus I; the term "almost" is used because it is probable that a pure type I virus cannot be maintained in tulip-bulb clones.

The following is a typical case history of selective segregation of Virus I and Symptom-type-1 from Farncombe Sanders Rembrandt. In 1930 inoculum from a striped leaf plant was injected into 103 healthy Farncombe Sanders by the standard hypodermic-needle method. Two months later, the inoculated clumps were dug, broken up into units of four to seven bulbs each and replanted—thus encouraging further segregation. Eighty-six of the 103 inoculations were positive (1931), and considerable variation appeared within each unit; these furnished abundant material for further selection. A strongly striped plant with almost white flowers was selected from these and used as a second source plant in 1931. This time, ten Clara Butt plants were injected with inocula from the white of the flower and ten similar plants with inocula from the leaf. In both cases infection was 100 %, but the leaf inoculum was noticeably milder in effect than that from the flower. The plant descendants from those inoculated with the white part of the flower were all dead by 15 April 1932, the time when they should have been coming into bloom, while a few of the plants inoculated from the leaf source produced small, misshapen, white flowers. All of our plants containing theoretically pure Virus I have died out during the season they exhibited symptoms.

Isolation of Virus II or colour-adding virus

Selective segregation and isolation of the colour-adding Virus II has proved more difficult than with colour-removing Virus I. Natural Symptom-type-3 segregates are comparatively rare, and even where a large population of diseased tulips has been available we have never found one that was entirely pure for this condition. Fairly pure segregations have been obtained in several instances, but the one which now (1936) seems to contain no trace of colour-removing virus has the following history. In 1930, inoculum from a naturally infected Vitellina exhibiting a typical break with mottled leaves was inoculated into nineteen King Harold and fifteen Bartigon. The transfer was 18/19 and 14/15, respectively. All of the King Harold positives were typical breaks for this variety, which *always* selfs. Twelve of the fourteen positive Bartigon were average breaks; the other two were entirely different, one tending towards a Symptom-type-1, the other being an almost perfect Symptom-type-3, with dark flower and unmottled leaves. In 1931

separate inoculations were made into Clara Butt from the dark flower of this plant and from its leaves. The flower inoculum gave (1932) a transfer of 9/10, all of which were perfect examples of Symptom-type-3. The leaf inoculum gave a transfer of 9/10, of which eight approached type-3, but the remaining one was a type-2 with mottled leaves, showing that in this case the Virus II had travelled faster and farther into the flower of the inoculum plant. In 1932 this selection of dark flower inoculum was repeated, this time inoculating Clara Butt and Professor Rauwenhof. The results were all type-3. This source has been maintained, and now in colour-removable tulips induces dark flowers which bear no trace of colour removal. The Virus II in the pure form does little damage to tulip plants and is therefore easily maintained in tulip clones.

The foregoing are specific case histories of tulip virus separation by selective inoculation. At the Oregon station a collection of what might be termed a "garden of segregates", derived from several varieties of tulips, has been maintained. It has been repeatedly shown that inoculations from different parts of a large clump which exhibits different symptoms will have different effects, indicating varying proportions of the viruses. Pl. VIII is an illustration of a case where obvious segregation has occurred.

SYMPTOMS PRODUCED BY THE VIRUSES

Table I, presenting the comparative effects of the two viruses, is based on several hundred inoculations made in the course of our investigations. This table must not be considered a description of tulip-breaking as it normally occurs. It depicts specific virus actions. The average broken tulip exhibits symptoms intermediate between these extremes. Tulip varieties representing all colours available have been used. In general, Virus I is a colour-removing virus, inhibits chlorophyll formation, greatly restricts growth, and is directly responsible for the recognition of tulip-breaking as a disease. Virus II or colour-adding virus has no effect on the ground tissue of the flower or on the ground colour, stimulates epidermal pigmentation, has no visible effect on the leaves, and has little effect on growth. Pl. IX shows the characteristic colour changes induced by these viruses in the pink flowers of the variety Clara Butt.

We have completed a property study of these two viruses but have not been able to separate them on a property basis. That they are really different is abundantly shown in their induced plant reactions. The subject of properties is reserved for a comprehensive paper on this subject, but the thought is here introduced that two viruses can be different,

antithetic, and even antagonistic—yet exhibit the same "physical" properties.

Table I

Symptoms induced in tulips by tulip Viruses I and II

Effect on	Colour-removing tulip Virus I	Colour-adding tulip Virus II
Plant size. All colours	Size of plant reduced 30–75%. Bulbs die out the second year	Size of plant reduced 10%
Leaves. All flower colours	Strong yellow-green stripe, *X*-bodies and virus type cell disintegration frequent. Leaves soon redden	No visible effect. No *X*-bodies
Stems	Purple pigment on stems reduced and rearranged	Epidermal purple pigments may become more conspicuous
Flowers, whites	Some clearing due to inhibition of ground tissue formation in areas which appear as hyaline spots referred to as "flecks"	Most white flowers turn pink or even strong red
Flowers, yellows	Do.	Some yellows turn red; others remain unchanged in appearance
Flowers, pinks	Turn white with only a trace of pink left. Pink remnants are darker than the original pink	All pinks tested turn a strong red. A trace of white is usually evidenced near the base. The colour "clots"
Flowers, purple shades	Some turn white with purple and dark red remnants; others white and darker purple	Turn very dark and beautiful purple. The colour "clots"
Flowers, reds	Most reds turn white with only a trace of red left; others darken (see Table III)	Turn very dark, almost black
Flowers, "blacks"	Darken and appear burned	Turn even blacker except on petal edges where white flecks appear

The relationship between tulip Virus I and tulip Virus II does not seem comparable to that between strains of a virus where the infection of a plant by one strain of a virus will protect the plant against infection by a related strain of the same virus. This infection interference is considered by Kunkel (1934) as an immunological reaction in the plant and thereby a measure of virus relationships; viruses which show infection interference are considered strains of the same virus, or at least of closely related viruses. In the course of these studies, Symptom-type-3 and type-2 plants have been successfully inoculated with type-I virus, so that the inoculates were completely changed. A type-II virus transfer into Symptom-type-1 has never been attempted, since type-I virus is known to be dominant. Virus terminology is as yet too unsettled to warrant a specific classification of the tulip viruses. They are different, but whether the difference is comparable to "species" or "strains" is a matter of

opinion. There is no evidence of immunization other than the *dominance* of Virus I; this would indicate, therefore, that the difference between these viruses is of a higher order than "strains".

SYNTHESIS OF NORMAL BREAKS

As stated above, two viruses were obtained through selective segregation from originally typical breaks. The separation of these viruses proved that typically broken tulips contain two viruses; it did not prove that such breaks could be induced without the presence of a third virus or factor. Proof that the two viruses in certain combinations are able to induce normal breaks was accomplished in two ways: (*a*) by injecting healthy tulip plants coincidentally with viruses from type-1 and type-3 plants; (*b*) by injecting healthy tulips with measured mixtures of the viruses in known proportions.

The symptoms induced by the two viruses, as recorded above, favour our interpretation that they are antithetic. Examination of the results from using measured mixtures of virus-bearing juices as inocula have yielded in terms of growth and colour responses, specific proof that these viruses are physiologically antithetic (see Table II).

Our first attempt at synthesis was made in 1931, using the fairly pure segregations available at that time. The virus-bearing juices diluted 1 : 10 were used as follows:

Virus I into nine Clara Butt induced 3/9, all Symptom-type-1.

Virus II into ten Clara Butt induced 4/9, all Symptom-type-3.

One part Virus I plus one part Virus II into nine Clara Butt induced 8/9, all Symptom-type-1.

One part Virus I plus three parts Virus II into nine Clara Butt induced 4/9, all Symptom-type-1.

Three parts Virus I plus one part Virus II into twelve Clara Butt induced 11/12, all Symptom-type-1.

The results of the test were disappointing because the type-1 virus proved completely dominant. At the same time these mixed virus inoculations were made, the 1 to 10 dilutions of each were injected *separately* into the same nine Clara Butt plants. This double inoculation induced 7/9 transfer of which five were Symptom-type-1 and two were Symptom-type-3 in character. The interpretation of these preliminary tests is that Virus I is dominant and that separate (coincident) inoculations may result in separate action of the viruses. Normal breaking was not duplicated.

Table II

Normal-broken tulip synthesis with virus combinations 1932 *inoculations; readings* 1933

Proportion and dilution	Ratio of dosages of viruses	No. plants inoculated	No. plants survived	No. positive	Distribution of Symptom-types induced	Total length in cm. of flower stems over no. of stems	Av. length in cm. of stems
One part of 1/100 Virus I to one part of 1/100 Virus II—combination inoculum	1 : 1	8	8	8	Two perfect type-1, five mild type-1, one type-2. Colour removing dominant	259/13	20
One part of 1/100 Virus I to ten parts of 1/100 Virus II—combination inoculum	1 : 10	6	6	5	Two type-1 and three type-2. Normal breaks typical	175/8	21
One part of 1/1000 Virus I to one part of 1/100 Virus II—combination inoculum	1 : 10	8	8	8	Two type-1, five type-2 typical normal breaks, one type-3. This mixture produced mostly normal breaks	634/17	37
One part of 1/1000 Virus I to ten parts of 1/100 Virus II—combination inoculum	1 : 100	7	7	5	No type-1, three type-2, two type-3	217/6	36
One part of 1/10,000 Virus I to one part of 1/1000 Virus II—combination inoculum	1 : 10	13	13	0	Inoculation failed. The transfer should have been at least 50 %. No explanation	—	—
One part of 1/10,000 Virus I to one part of 1/100 Virus II—combination inoculum	1 : 100	7	7	4	Virus II entirely dominant. Leaf mottle absent. There were nine flowers; only four showed a trace of colour removal. Symptom-type-3	222/7	32
One part of 1/10,000 Virus I to one part of 1/10 Virus II—combination inoculum	1 : 1000	7	7	5	All type-3	208/6	35
Check—1/100 Virus I only	—	5	5	5	All type-1, plants were killed down. No flowers produced	—	0·0
Check—1/100 Virus II only	—	8	8	8	All good type-3, all flowers selfed	—	—
1/100 Virus I and 1/100 Virus II separate inoculations	1 : 1	6	6	6	Five type-1, one type-2. Type-1 dominant	—	—
1/10 Virus I and 1/100 Virus II separate inoculations	10 : 1	7	7	7	Two type-1, four type-2 with type-1 tendency. One type-2 dark break	—	—
1/100 Virus I and 1/10 Virus II separate inoculations	1 : 10	12	11	10	Three type-1, six type-2 and one type-3		

Total length of the flower stems of all the type-1 inoculates 353/17. Average 20·7 cm.
" " " 2 " 805/24 " 33·5 "
" " " 3 " 526/15 " 35·0 "
The total length of flower stems of the comparable plants that remained healthy 1662/39.
Average 42·6 cm.

Note. The plants inoculated with type-1 alone produced no flowers and hence are not listed in the above table of lengths of stems; the length was 0.

Using the excellent virus segregates available in 1932, a comprehensive series of combination inoculations was made. These were tabulated in Table II. One source plant was used for each virus. The coincident inoculations were made shortly after extracting the virus-bearing plant juices. The proportioned mixtures were allowed to stand several hours before inoculating so that possible interaction between the viruses might be initiated before they had a chance to begin development within plant tissues to which they were subsequently exposed during infection processes. Previous experiments had shown that the infectiousness of these viruses when inoculated separately is not materially affected by ageing within a period of 2 days.

The distribution of types induced by combination inoculations of different proportions of the viruses must not be taken too literally because even when pre-mixed inocula are injected there is always a chance for selective infection. The results, however, are considered indicative of the proportion of the two viruses necessary for typical tulip-breaking. The exact virus proportions constituting the physiological balance of the typical break (type-2) cannot be exactly stated until these viruses can be isolated and quantitatively measured. In these experiments typical breaks were synthesized from double-virus inoculations. If the proportions of diluted virus-bearing juices inoculated are true criteria, then a typical break may be considered an expression of tulip Viruses I and II where the concentration of the colour-adding II is at least 10 times that of the colour-removing I. This conclusion is based on the pink tulip, Clara Butt. The proportions of Viruses I and II required for the production of typical breaks in other varieties have not been determined, but it is probably the same since inoculation with juice from typical type-2 Clara Butt plants induces typical type-2 breaks in other varieties.

The dominance of the colour-removing Virus I seems proved not only by the specific inoculations here recorded but by the tendency of plants with a preponderance of this virus to segregate out during the propagation of commercial Rembrandts.

The strongest proof of the antithetic intervirus reaction which plays a role in the synthesis and stabilization of the average tulip break is the relative growth performance of plants which have received individual inocula compared with those which have received mixed inocula. In every variety of broken tulips that we have studied the type-2 plants are invariably larger than the type-1 segregates (or inoculates). The presence of the Virus II where Symptom-type-2 is present reduces the shock of Virus I and limits its ability to inhibit growth. This effect is

reflected in the height data of Table II, but the effects of selective infection, when combined inocula are employed, cannot be circumvented. The "length of stem" data are measurements to the base of the flower. The comparable height of the five plants which received Virus I must be recorded as zero, since the plants were dead by blooming time and produced no flowers. It is significant that all the mixed virus inoculations, including those mixtures consisting of equal proportions, produced flowers, even in cases where flower and leaf types showed that the Virus I had remained completely dominant. There is good agreement between long stems and the presence of Virus II except in the height class 37 cm. In this case where Viruses I and II were present in dilutions of 1 : 1000 and 1 : 100, respectively (ratio of I to II is 1 : 10), the departure from the expected stem shortening due to Virus I must be attributed to experimental uncertainty at this dilution. Since these experiments involve a 2-year planting and the records are taken during the second year, the extreme growth variations, which might have resulted from differences in sizes of bulbs planted, are largely eliminated, thereby making the stem length differences attributed to virus action relatively more significant.

The experiments of 1932–3 proved our contention that normal tulip-breaking, as exemplified in pink varieties, results from the interaction of two viruses. In the course of a study of properties which has just been completed, we hoped to obtain these viruses in purer form and repeat these synthesis tests. This quest was unsuccessful. We have been able to separate them only by means of the segregation method described above.

The behaviour of red-flowered tulips

A confusing point relating to tulip virus analysis is the fact that some dark red tulip varieties never break white or form the pattern which Hughes (1934) calls "full break". In these red varieties so far tested the factor for increased redness is fixed within the plant so that the presence of either virus is indicated by added colour; the plant, not the virus, controls the type of break. These red effects, for which some hidden factor within the plant is responsible, must not be confused with the reddening brought about by the colour-adding Virus II alone. In these unorthodox red varieties Virus I induces characteristic leaf mottle, but Virus II brings about no recognizable effects in the leaves. The type or types of virus present in these reds can be surmised by examination of the leaves and flowers, and proved by inoculation into pink varieties.

The similarity of physical properties of two viruses so different in physiological effect suggested that the factor for colour addition might

not be a virus but a plant-borne factor related to that which causes some red tulips never to break white or assume the full-break pattern. Cross inoculations were made from virus infected, non-red and red tulips into healthy red varieties with the purpose of finding some recognizable plant character which could be correlated with what may be termed the ability of most red tulips to break into white and red patterns, that is, to assume the full break condition. A selected list of these inoculations is given in Table III. In every case the inoculum used should have induced a full break had not some of the reds (those listed as selfed) contained a factor limiting breaking. No recognizable plant characteristic has been found capable of serving as an indicator of the type of break induced.

Table III

Tabulation of inoculations on red tulips. Inoculated plant record

Virus source variety	Symptom type	Variety inoculated	Year	No. plants inocu- lated	No. survived	No. positive	Type of break
Farncombe Sanders	2	Allard Pierson	1930–1	5	5	5	Self
Solfatare	2	Bartigon	1930–1	20	19	17	Full
Avis Kennicott	2	,,	1930–1	20	20	15	,,
Vitellina	2	,,	1930–1	20	16	14	,,
White Queen	2	,,	1930–1	20	19	16	,,
Clara Butt	2	,,	1932–3	7	7	7	,,
,,	1	,,	1932–3	8	4	4	,,
,,	2	City of Haarlem	1932–3	15	13	9	Self
Diana	2	Farncombe Sanders	1930–1	20	20	2	Full
Prince de Ligny	2	,,	1930–1	20	20	9	,,
Feu Brilliant	2	,,	1930–1	20	17	9	,,
Sieraad van Flora	2	,,	1930–1	20	16	6	,,
Bronze Queen	2	,,	1930–1	20	16	6	,,
La Tulipe Noire	2	,,	1930–1	20	18	3	,,
Professor Rauwenhof	2	,,	1930–1	20	13	13	,,
Farncombe Sanders	2	,,	1930–1	5	5	5	,,
Clara Butt	2	,,	1931–2	40	39	38	,,
,,	2	,,	1932–3	9	9	8	,,
,,	1	,,	1932–3	10	8	7	,,
,,	2	Feu Ardent	1932–3	16	9	8	,,
Farncombe Sanders	2	Feu Brilliant	1930–1	5	5	5	,,
Clara Butt	2	Harry Veitch	1932–3	12	10	8	Self
,,	2	Jacob Maris	1932–3	16	14	7	,,
Solfatare	2	King Harold	1930–1	20	19	17	,,
Avis Kennicott	2	,,	1930–1	20	16	15	,,
Vitellina	2	,,	1930–1	20	19	18	,,
White Queen	2	,,	1930–1	20	19	17	,,
Clara Butt	1	,,	1932–3	8	8	8	,,
,,	2	La Merveille	1932–3	8	8	8	Full
,,	2	Lucifer	1932–3	15	14	3	,,
,,	2	Prince Albert	1932–3	16	15	9	Self
,,	2	Prince of the Netherlands	1932–3	12	11	6	Full
,,	2	William Pitt	1932–3	16	15	14	,,
,,	1	,,	1932–3	8	7	6	,,
,,	1	La Tulipe Noire	1932–3	6	4	4	Self
Farn. Sanders Rembrandt	—	,,	1930–1	20	18	16	,,
Farncombe Sanders	2	,,	1930–1	5	5	5	,,

The red varieties so far tested which have proved incapable of full breaking are City of Haarlem, Allard Pierson, Harry Veitch, Jacob Maris, King Harold, Prince Albert, and the so-called black tulip, La Tulipe Noire. Allard Pierson and King Harold have been subjected to special study. We have injected both viruses separately and mixtures of Viruses I and II into these reds and then made return inoculations into pinks to determine whether the procedure would increase the factor for darkening. Passing the viruses through these special reds had no modifying effect on either virus. Moreover, in a comprehensive variety and colour test it was shown that no tulip variety tested, including several dark reds and La Tulipe Noire, had any direct effect on the inoculum.

The passage of a balanced virus mixture through these special reds had no effect on the mixture; the factor for inherent redness could not be picked up during subinoculation. A further check on this interpretation was made possible by inoculating Clara Butt with juice from healthy reds of the King Harold group. The flowers of the inoculates could not be distinguished from their sister-bulb checks.

The variety Allard Pierson[1] is an excellent example of a red tulip which does not show a trace of white even when inoculated with a purified Virus I. A test was made in 1935–6 to determine whether the juice of this tulip would behave like an antigen to the colour-removing virus. For this purpose juice from an average broken Clara Butt was used as inoculum representing a natural physiologically balanced mixture of Viruses I and II. The Clara Butt juice was filtered through cloth, cleared by allowing to stand and by decanting, then diluted with sterile distilled water as indicated below. The juice from healthy Allard Pierson was used undiluted. The mixtures of healthy juice and diluted virus-bearing juice were kept at a temperature of about 9° C. for 16 hr. before injecting them into healthy Clara Butt plants on 2 May 1935. In the tabulation below, "A.P." signifies healthy Allard Pierson juice and "C.B." refers to Clara Butt juice bearing an average virus mixture. The readings are for April 1936.

One c.c. C.B. diluted 1 : 20 plus 5 c.c. A.P. induced 11/15 average break
One c.c. C.B. diluted 1 : 100 plus 5 c.c. A.P. induced 10/12 average break
One c.c. C.B. diluted 1 : 200 plus 5 c.c. A.P. induced 9/13 average break
One c.c. C.B. diluted 1 : 1000 plus 5 c.c. A.P. induced 2/13 average break
Check C.B. juice diluted 1 : 50. Induced 11/12 average break
Check A.P. juice diluted 1 : 10. Induced 0/15 unbroken

[1] The writer has been informed on good authority that Allard Pierson listed as crimson maroon (Darwin) by Krelage in 1926 is a reselection of the old variety, Mrs Allard Pierson.

A thorough examination of individual flowers and leaves of this group of inoculates showed that the Allard Pierson juice had no modifying effect whatever. There was no significant variation in the degree of colour removal. It should be noted that in the highest dilution used, the virus-bearing juice was diluted 1 : 5000 when added to 5 c.c. of Allard Pierson juice, a proportion that would have given dark flowers were this factor which inhibits red pigment removal able to function as does Virus II, the colour-adding virus.

The conclusion from these observations on these special reds is that Virus II is entirely distinct from the factor for self-breaking in these dark red varieties. Moreover, the obscure factor for self break in these reds does not seem to be a virus and is not separable from the plants which contain it. It is certainly not the same as Virus II which enters into the average break and has the property attributes of a true plant virus.

INTERPRETATION OF TULIP-BREAKING INDUCED BY ANTITHETIC VIRUSES

The above data point to two viruses being present within the typically broken tulip plant. The viruses are the initial causal agents. The effect of the viruses is a function of proportion and of certain limiting factors within the plant. These limiting factors which regulate what may be termed the symptom display contingent to the viruses may be grouped as follows:

(*a*) The presence or absence of precursors capable of forming red pigments in the epidermis of white and yellow flowers.

(*b*) The presence or absence of substances capable of forming red pigments in flower epidermis which is normally purple.

(*c*) The presence or absence of factors which inhibit colour removal by Virus I.

(*d*) In some Triumph tulips a factor or factors are present which prevent viruses from changing the original colour pattern. In these cases the usual pattern merely shrinks in size, without yielding to differential colour changes.

The solution of these plant factors will lead to a biochemical analysis of formative anthocyanins and their determinants on the one hand, and to a genetical analysis of tulip varieties and groups on the other. Complete analysis portends difficult but fundamental research. At present we are initiating a more extensive study to determine the varietal range

of distribution of the factor, or factors, which inhibit the removal of red pigments.

The broken tulips known commercially according to the colour and group of their healthy prototype as Rembrandts, Bybloemen, Bizarre, etc., represent responses to physiologically balanced mixtures of two viruses. Many of these named varieties are of reasonably vigorous growth, though not quite equal to their prototypes, some of which are called "breeders" because of their vigour. The fact that these commercial broken tulips continue to grow and reproduce led to the old theories of "rectification" and the assertion that tulip-breaking is not a disease. The theory was that a broken clone became "rectified" with age. A further proof that such broken tulips represent a balanced virus condition was shown by deliberately overbalancing one of these established varieties by inoculating typical plants with Virus I. In this experiment, nine plants of the Bybloemen Violet Wodan were inoculated; five of these changed from the dark type of commerce to dominantly white flowered forms with only a remnant of purple. The average height of the flower stalks of the inoculates was 28 cm.; that of the check Bybloemen was 43 cm. This test is particularly significant since a 5-year record on this variety shows that it is one of the most stable of the broken Dutch breeders that we have kept under observation. It would seem, however, that the old term "rectification" may be justified technically as a descriptive term of the process through which newly infected tulips go while their virus burdens appear to adjust themselves into a physiological balance.[1]

In the present article, relatively little attention has been given to selfing versus clotting. These symptoms are inter-related between virus effects and plant factors. For example, those reds in which a full break cannot be induced tend to clot. Average mixtures of viruses tend to induce self-breaks, the term being used to refer to the condition known as "feathering". As the virus proportion approaches the pure II condition, clotting becomes more pronounced, and inoculates which receive the pure II virus exhibit clotting only. The appearance of the leaf is, however, a more reliable criterion of the dominant virus type than the pattern of the added colour.

With these viruses singled out and isolated in plant segregates and with an understanding of the limiting factors that are functions of tulip

[1] A further significance will be given to the term in a paper now in preparation which will prove that the probable ultimate source of the broken tulip is the group of bulb-perpetuated Chinese lilies.

varieties, one may proceed literally to make broken tulips to order. But even well-balanced, average-broken tulips are a menace to other tulips because of the danger of segregation and selective action of Virus I. Alone it is a killing virus. The Virus II, however, when pure deserves consideration as a possible tool for the creation of truly beautiful flowers. This virus adds to purples and pinks beautiful darker shades without materially reducing vigour. The patterns are always clotted and sometimes beautiful. This suggests the question: Can induced patterns be controlled?

As a result of observation of more than 5000 tulips broken with known or recorded inocula, the following general observations seem warranted. If a mixture of Virus I and II is taken from a plant showing a typical symptom complex (this connotes a balanced virus mixture) and inoculated into 100 tulips, 99 out of the 100 may be expected to show an average break and a similar pattern. The remaining plant may show selective segregation towards the type-1 or type-3 conditions. The tulips in the group will assume the same general pattern, but this pattern will usually not closely resemble that of the inoculum source plant. We have never been able to make a conspicuous pattern transfer even where inoculum and inoculates represented the same variety of tulip. The foregoing statements relate to physiologically balanced virus mixtures. When unbalanced virus mixtures are used, the induced colour patterns vary greatly in appearance. When a source plant containing a pure Virus I is used, there is usually no flower and hence no pattern. When a plant containing pure Virus II is used, there is better agreement between the patterns of the inoculum plant and the ones inoculated. The pattern of the flower colours induced remains uncontrollable.

SUMMARY

The term antithetic is suggested for viruses which are usually associated and which are physiologically antagonistic.

Tulip breaking results from the interaction of Tulip Virus I which inhibits flower and leaf colour and Tulip Virus II which adds flower colour but has no visible effect on chlorophyll distribution.

The established commercial broken tulips contain physiologically balanced mixtures of these two viruses.

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EXPLANATION OF PLATES VIII AND IX

PLATE VIII

A Farncombe Sanders plant was inoculated with a tulip virus mixture in 1929. In 1930 it developed a typical break. Bulbs were then transplanted and the subsequent plants left undisturbed. In 1932, virus segregation occurred as indicated in this photograph.

PLATE IX

Fig. 1. A Clara Butt flower produced by a plant where tulip Virus I (colour removing) is present and dominant.

Fig. 2. A Clara Butt flower produced by a plant where both Virus I and Virus II are present and are "physiologically" balanced.

Fig. 3. A Clara Butt flower produced by a plant where tulip Virus II (colour adding) is present.

Fig. 4. Flower of healthy Clara Butt tulip.

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THE RELATION BETWEEN POTATO BLIGHT AND TOMATO BLIGHT

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(With Plate X)

Epidemics of blight (*Phytophthora infestans* (Mont.) de Bary) on outdoor potato and tomato crops are frequent in Jersey. The early potatoes, variety International Kidney, are planted in January and February and blight usually appears in April; in May and June the crop is in full growth and disease may become prevalent in either month. The young tomato plants, varieties Sunrise and Devon Surprise, are transferred from boxes to the fields in May and June, and, except on rare occasions when the plants are attacked in the boxes, blight appears after the crop is well established in the open. In experiments where potatoes were interplanted with tomatoes in the field in spring, the former were almost killed with disease before the tomatoes were attacked. Under field conditions blight appears first on the potato crop each year.

Most growers assume that the disease passes from the potato to the tomato and, in order to prevent this, diseased potato crops in the neighbourhood of tomatoes are sometimes destroyed. The validity of this assumption has been tested in the present investigation. It may be noted that the symptoms of blight on outdoor tomatoes are similar to those of potato blight.

Experimental methods

Sources of inoculum. Pure cultures were not used. Fresh diseased tissue was taken and, if necessary, was kept in a moist chamber for 1 or 2 days to encourage sporulation. It was then floated on tap water to obtain a suspension of conidia which was used immediately for inoculation purposes. Diseased tissue was obtained from: (*a*) the early glasshouse potato crop in January, February or March, (*b*) the early outdoor potato crop in spring, (*c*) the late outdoor potato crop in summer and autumn, (*d*) the outdoor tomato crop in summer and autumn.

Inoculation experiments. Young potato plants (variety International Kidney) and tomato plants (varieties Sunrise and Devon Surprise) in pots were used, and also large detached leaves with the petioles in water.

The results obtained with detached leaves are not recorded since they were similar to those obtained with pot plants. Both varieties of tomato are susceptible to blight.

The suspension of conidia was applied to the lower surfaces of the leaves with an atomizer, and the plants were kept in a very moist chamber in the laboratory throughout the experiment. In all the trials, blight taken from either host was tested on both potatoes and tomatoes so as to compare the symptoms and to ascertain if the environmental conditions were suitable for infection. Severe infection was established in 5–7 days but in doubtful cases the experiments were continued for 10–14 days. Controls were sprayed with tap water from the atomizer before the latter was used for the spore suspension; the controls were placed in the same chamber as the inoculated plants.

Inoculations with blight from early glasshouse potatoes

These experiments were carried out in January, February or March, a period when no outdoor tomatoes are being grown. The spore suspensions were obtained from diseased potato leaves of the variety International Kidney. Inoculated potato plants or leaves always developed symptoms of ordinary potato blight, while inoculated tomato plants reacted as follows.

In 1935 two experiments were made, using blight from two distinct glasshouse nurseries. In one trial the plants remained healthy; in the other each plant developed a few spots of typical blight with abundant sporulation.

In 1936 the tomato plants were severely infected with potato blight 7 days after inoculation.

In 1937 two experiments were made, using blight from the same glasshouse but collected in January and February respectively. In all cases typical blight infection was established on inoculated tomato plants, but the number of lesions was small.

The results show that, in four out of five experiments, symptoms of ordinary blight developed on tomato plants inoculated with blight taken from glasshouse potatoes, in January, February and March.

Inoculations with blight from early outdoor potatoes

Spore suspensions were obtained from the very early outbreaks of blight discovered in April or May on the outdoor potato crop, variety International Kidney. At this time little, if any, tomato blight was likely to be present in the island. All inoculated potato plants became

severely infected in 5–7 days. On tomatoes the following results were obtained.

In 1933 six experiments were made, and of the thirty plants used twenty-nine remained perfectly healthy. One plant developed several small (no more than $\frac{1}{4}$ in diam.) black spots on the leaves; the spots did not enlarge and kill the foliage and no definite sporulation was observed.

In 1934 all six inoculated plants remained healthy.

In 1935 one plant remained sound but four developed numerous small black spots on the foliage similar to those of 1933 except that a little sporulation occurred. This was the first record of definite infection of tomato plants with blight from the early potato crop.

In 1937 two experiments were made and six plants remained free from infection, while four developed a total of seven spots similar to those observed in 1933 and 1935; no sporulation occurred.

The results show that, under the experimental conditions, blight from the potato infected the potato severely in 5–7 days, but often failed entirely to infect the tomato. Where infection of the tomato was established, the symptoms produced were quite unlike those found on potatoes and tomatoes in the field and were similar to those described by Berg (1926).

To determine if potato blight would infect tomatoes under field conditions, sprouted seed potatoes, variety International Kidney, were planted in April 1935, and when the shoots appeared above the ground on 11 May, forty-five young tomato plants were set out in rows between the potatoes. All the plants were left unsprayed. On 19 June blight appeared on the potatoes which were now fully grown and in contact with the tomatoes; on 27 June the potatoes were severely diseased, but only one spot of blight had appeared on the tomatoes; on 14 July the potato haulms were dry and shrivelled and very few diseased spots were present on the tomatoes. These tomatoes did not develop any of the small, black spots described in the above laboratory experiments.

In 1936 similar results were obtained. In each year the rapid destruction of the potatoes showed that conditions were favourable for blight. Ample time was allowed for the tomatoes to become infected, but they remained practically healthy despite the fact that they were not sprayed. Yet under field conditions tomato crops are rapidly destroyed by blight, and experience has shown that it is necessary to spray tomatoes every 7–10 days throughout the season.

The results of the laboratory and field experiments indicate that early

potato crops affected with blight are not nearly so dangerous to neighbouring tomato crops as is commonly assumed.

Inoculations with blight from late outdoor potatoes

These experiments were made in summer and autumn when both potato and tomato blight are present outdoors. The spore suspensions were obtained from diseased leaves of Kerr's Pink or Great Scot, except in 1936 when leaves from "volunteer" potato plants were used. Blight developed quickly on all inoculated potato plants; on tomatoes the following results were obtained.

In 1933 all five plants developed many small black spots. In addition, a single ordinary blight lesion, with abundant sporulation, was produced on each of two leaflets of one plant and on one petiole of another plant. This was the only time during the investigation that two distinct types of lesions were obtained on the same plant. The controls remained healthy.

In 1934 each plant developed numerous small black spots which remained small and produced many spores. Potato blight collected in autumn 1934 was grown throughout the winter on potato leaves in the laboratory by means of weekly inoculations of fresh tissue. In March and April 1935 this blight was used to inoculate tomato plants, and the results were similar to those obtained in autumn 1934.

In 1936 spore suspensions were made from diseased leaves of "volunteer" potato plants found outdoors in the autumn. Inoculation of tomato plants produced numerous small black spots which developed very few spores.

The results show that successful infection was established on all tomato plants inoculated with blight taken from outdoor potatoes in autumn. Typical tomato blight symptoms were not obtained except on two plants in 1933.

Inoculations with blight from outdoor tomatoes

In these experiments the spore suspensions were taken from diseased seedlings, leaves, stems, and fruits of outdoor tomatoes, varieties Sunrise or Devon Surprise. At this time of the season blight is usually present on both potato and tomato crops in the field.

Ten experiments were made over a period of 4 years and, in every case, typical blight symptoms developed quickly on all inoculated tomatoes and potatoes. The results agree with those recorded by Giddings & Berg (1919) and by Berg (1926).

Further trials were made to determine if blight from the infected potatoes in the above experiments would attack tomatoes. Ordinary blight symptoms developed in every case.

The results show that blight from the leaves, stems, and fruits of the tomato infected the potato, and that the blight so produced on the latter passed back readily to the tomato.

SUMMARY AND CONCLUSIONS

1. In Jersey, potato and tomato crops are grown in close proximity in the open, and both suffer from severe attacks of blight (*Phytophthora infestans*). The disease appears first on the potatoes, and most growers assume that it passes from this crop to the tomatoes. To test this assumption, potato and tomato plants have been inoculated, using blight from either host plant.

2. In four out of five experiments, ordinary blight lesions developed on tomatoes inoculated with blight from glasshouse potatoes in January, February and March. The result suggests that tomato plants for the outdoor crop are liable to contract blight, if, as is often done, they are raised in glasshouses where diseased potatoes have just been, or are being, grown.

3. Blight from the early outdoor potato crop often failed to infect tomatoes. Where infection did occur typical symptoms of blight were not produced. When, under field conditions, early potatoes were interplanted with tomatoes, the former were quickly killed by blight while the latter remained almost healthy. The evidence indicates that early potato crops affected with blight are not so dangerous to neighbouring tomato crops as is commonly assumed.

4. Successful infection was established on all tomatoes inoculated with blight taken from outdoor potatoes in autumn, but ordinary blight lesions were not produced except in one experiment.

5. Blight taken from the leaves, stems, or fruits of the tomato infected the potato, and the disease so produced in the latter passed back readily to the tomato. This indicates that diseased tomato crops may be a serious menace to neighbouring potato crops.

6. The results of numerous inoculation experiments support the view that more than one strain of *Phytophthora infestans* exists.

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EXPLANATION OF PLATE X

Fig. 1. Tomato plant 14 days after inoculation with blight from potato.

Fig. 2. Tomato plant 7 days after inoculation with blight from tomato.

(*Received* 20 *August* 1937)

Fig. 2.

SMALL.—THE RELATION BETWEEN POTATO BLIGHT AND TOMATO BLIGHT (pp. 271-276)

INFECTION EXPERIMENTS WITH *CLADOSPORIUM FULVUM* COOKE AND RELATED SPECIES

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(With Plates XI and XII and 16 Text-figures)

CONTENTS

I. INTRODUCTION

PREVIOUS experience of the leaf-mould disease of tomatoes (*Lycopersicum esculentum* Mill.) caused by *Cladosporium fulvum* Cooke indicated that further study of the pathogenic behaviour of the causal organism was desirable (Bond, 1936); the taxonomic position of the fungus also invited a comparison with the behaviour of certain other species of the genus. *C. fulvum*, which appeared to be confined to the single host species *Lycopersicum esculentum*, nevertheless exhibited only a very slight degree of physiologic specialization (Langford, 1937). This, coupled with its ability to develop as a saprophyte, although rarely found as such under natural conditions, placed it in an interesting position between the obligate parasites, such as the rusts, showing a high degree of specialization and incapable of saprophytic development, and the relatively unspecialized, facultative parasites, such as *Botrytis* spp., which appeared

equally well fitted to a saprophytic existence as to parasitism. *Cladosporium herbarum*, according to recent opinions (Bennett, 1928; Bockmann, 1933), should be regarded as almost exclusively saprophytic, thus providing a strong contrast to the behaviour of *C. fulvum*. Various strains of *C. herbarum* were available from among the original isolations of "sooty mould" occurring on tomato foliage already infected by the leaf-mould disease. The choice of *C. cucumerinum* Ell. & Arth. as a third species for investigation was determined partly by its occurrence as a pathogen of cucumber (*Cucumis sativus* L.), apparently under conditions similar to those favourable to the development of *C. fulvum* on the tomato. As the following account will show, *C. cucumerinum* proved to be in many respects intermediate between the other two species investigated.

II. Materials and methods

Host plants

Varieties of tomato (Lycopersicum esculentum *Mill.*).

Previous work by the writer (Bond, 1936) had shown that out of a dozen common varieties "Giant Red" was the most highly susceptible, while "Stirling Castle" and "Maincrop" were relatively resistant. These three varieties were accordingly grown, seed being obtained from the same firms as formerly.

Small-fruited tomatoes.

Varieties obtained commercially included "Cascade" and the previously described "S.F. 3" type of currant tomato (Bond, 1936), identified as *L. pimpinellifolium* Mill. (= *L. racemigerum* Lange). Varieties and species obtained from other sources were as follows:

From the Royal Botanic Gardens, Kew: *L. racemigerum*.

From the University Botanic Garden, Cambridge: *L. racemigerum* (Cambridge), *L. racemigerum* Lange (from Copenhagen, No. 216–35), *L. Humboldtii* Dun. (from Copenhagen, No. 130–36).

From the John Innes Horticultural Institution: *L. pimpinellifolium* (from E. W. Lindstrom, No. 1673–3), *L. racemigerum* (Nos. 81/34 and 20/35 of Dr Sansome's collection).

The nomenclature of the currant tomatoes is discussed below (p. 287).

Other plants.

These included members of the Cucurbitaceae (Carter's "Telegraph" cucumber, *Cucumis sativus* L., also *Bryonia dioica* Jacq.), various other "inappropriate" hosts of garden origin such as asters (*Callistephus* sp.),

red and yellow snapdragon (*Antirrhinum majus* L.), and ten-week stock (*Matthiola* sp.), and the following solanaceous plants: *Solanum nigrum* L., *S. dulcamara* L., *S. melongena* L., *S. capsicastrum* Link., *Nicotiana tabacum* L. (var. "White Burley"), *N. glutinosa* L., *N. sylvestris* Speg. & Comes., *Datura stramonium* L., *D. meteloides* DC., *Atropa belladonna* L., *Hyoscyamus niger* L., *Browallia viscosa* H.B.K., *Schizanthus grahamii* Gill. In addition, the writer is indebted to Dr d'Oliveira for the following varieties of *Capsicum* grown commonly in Portugal: "Pimentos da America" (*C. annuum* L.), "Pimentos Morrones, espanhois" (*C. annuum* L., b. *grossum* Willd.), "Pimentos do Chile (Malaguetas)" (*C. frutescens* L.).

All host plants were grown from seed in potting soil supplied from the University Botanic Garden. Adequate glasshouse accommodation was available, and no difficulty was experienced in maintaining a supply of seedling plants at all times of the year except for a period of 6 or 7 weeks from the middle of November onwards.

Fungal cultures

The original cultures of *Cladosporium fulvum* (1/33) and of *C. herbarum* (2/33) were isolated in October 1933 from diseased tomato foliage obtained in the neighbourhood of Reading. Fresh single-spore isolations were made from this material at the commencement of the investigation in October 1935. Cultures received or isolated subsequently to this date were numbered as follows:

C. fulvum: 4/35 (from the Centraalbureau voor Schimmelcultures, Baarn), 10/35 (isolated from diseased tomato foliage sent from the Experimental and Research Station, Cheshunt), 11/35 (from diseased material sent from the States' Experimental Farm, Jersey).

C. cucumerinum: 2/35 (from Baarn); 1/36 (from Cheshunt).

Stock cultures of these fungi were grown on slants of Dox's medium or quaker-oat agar, incubated at 25° C. and at laboratory temperature.

Experimental methods

Inoculation experiments were carried out in the glasshouse on potted plants and in the laboratory on detached leaves in Petri dishes. The controlled environment chamber modified by Wilson (1937) was also used. For each experiment a minimum of four plants or leaves was employed, including one uninoculated control. Potted plants after incubation under bell-jars were kept free from contamination by placing them beneath light, cellophane-covered frameworks. Foliage infected

with *C. fulvum* was removed at the end of each experiment and stored in a refrigerator at 5° C. Under these conditions, the spores were found to retain their vitality for long periods. Material was inoculated by spraying with a suspension of spores in water or nutrient solution or, in some experiments, by local application of small drops of spore suspension or portions of the fungus colony, held in place by a fleck of absorbent cotton-wool. Spores of *C. fulvum* were obtained from the stored, infected foliage or from colonies on quaker-oat agar. For the other two species, colonies on Dox's agar were preferred. To prepare the spore suspensions the spore-bearing material was placed in a filter funnel and sprayed by means of an atomizer.

The details of penetration were observed in fixed material, using two different methods of examination. For sections the material was fixed in Carnoy's fluid, cleared in xylol and embedded in paraffin. Sections were cut 10–12μ. thick and were stained in carbol thionin blue and differentiated in orange G according to the method described by Stoughton (1930). Photomicrographs of these preparations were taken with the aid of a Wratten "B" (green) filter. Unsectioned material was cleared in lacto-phenol and stained in cotton blue by Pady's (1935) method. In some instances, adequate clearing was obtained only after an additional treatment with a saturated aqueous solution of chloral hydrate. Cemented mounts in lacto-phenol were found to be reasonably permanent. The preparations were photographed with the aid of a Wratten "F" (clear red) filter. The use of the lacto-phenol method permitted the examination of a much larger quantity of material than would otherwise have been possible. Moreover, the actual extent and distribution of the mycelium within the leaf could be recorded and a comparison made of the rate of penetration in different varieties and under different conditions.

III. *Cladosporium fulvum*

By most workers, the leaf-mould disease of tomatoes has been investigated largely from the practical standpoint of the discovery of control measures and the development of resistant varieties. The extensive literature dealing with the results achieved along these lines is summarized in the recent publications by Chamberlain (1932) in New Zealand, by Sengsbusch & Loschakowa-Hasenbusch (1932) in Germany, by Guba (1936) in the United States, and by Langford (1937) in Canada. The more theoretical issues of importance to the present investigation are discussed together with the relevant experimental work.

(1) *Experiments with varieties of tomato*
(Lycopersicum esculentum *Mill.*)

(*a*) *Symptoms.*

Susceptible varieties. On "Giant Red", under suitable conditions, the infection is characterized by the abundant and rapid development of the fungus and the early commencement of sporulation. Infected foliage ultimately shrivels from the apex and margins inwards, becoming covered by a dense mass of spores (Pl. XI, fig. 1).

Resistant varieties. Resistance is expressed by the slower development of infection and by a reduction in the intensity of sporulation. In "Stirling Castle", the infected areas appear smaller than in "Giant Red" and remain distinct, being often localized towards the apex of the leaf. In "Maincrop", sporulation is very scanty and is confined at first to the centre of sharply circumscribed yellow blotches, where it marks, presumably, the original points of infection (Pl. XI, fig. 2). Later, as the yellowing becomes more general, the spores are produced over a wider area. In the final stage shrivelling occurs at the centre of the original blotches and also from the apex and margin inwards, as in "Giant Red". This condition, however, occurs comparatively rarely and involves only one or two leaves of the plant at a time (Pl. XI, fig. 3).

In both types the progress of infection was recorded by a numerical method adapted from that employed originally by Small (1930). For every leaflet examined, points were awarded for the visual estimate respectively of the percentage of the total area of the leaflet infected and the average degree of sporulation, as follows:

For the percentage area infected:

Up to and including 20%	1
More than 20%, up to and including 40%	2
More than 40%, up to and including 60%	3
More than 60%, up to and including 80%	4
Above 80%	5

For the degree of sporulation:

"Incipient" (no evident aerial mycelium)	1
Mycelium only (no evident sporulation)	2
Slight sporulation (pale brown)	3
Medium sporulation (dark brown)	4
Intense sporulation (dark to blackish purple) ...	5

The two marks were multiplied together to give a maximum value of 25 or (multiplied by 4) 100%. From three to seven leaflets were recorded

on each leaf, and the total score was expressed as an average percentage value for the leaf as a whole. The same number of leaves was recorded in each plant. The results of a typical experiment involving all three varieties are presented in Table I. As the figures show, the varieties were clearly distinguishable. Infection was most severe in the middle region of the plant. At the end of the experiment the plants had grown away from the infection, leaves which to a large extent escaped infection previously becoming fully susceptible on reinoculation. The resistance of the older basal leaves was noticeable. On such leaves the early symptoms of the disease were admittedly less recognizable, but sporulation was invariably reduced. The data obtained by this method of recording infection were amenable to statistical analysis. The method was found suitable for investigating the effect of manurial treatment on the incidence of the disease. It was also used in an attempt to distinguish between the four strains of the fungus available, but with negative results. Langford (1937) has recently described from Canada a new physiologic race characterized by its extreme virulence towards varieties normally resistant to infection. Apparently, it is not known to occur in this country, in which the behaviour of resistant varieties such as, for instance, "Stirling Castle", appears fairly consistent.

Table I

Percentage intensities of infection: averages for three plants

	Leaf No.														
	(Lowest) I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	(Uppermost) XIV	XV
							"Giant Red"								
Weeks															
2	—	—	—	—	—	—	2	4	4	3	5	2	1	—	—
3	7	7	16	43	42	60	54	63	58	41	33	23	13	2	—
4	7	34	48	89	92	80	96	99	97	77	56	35	26	7	—
5	7	34	48	93	100	100	100	100	100	83	61	44	30	8	2
							"Stirling Castle"								
2	—	—	—	—	—	—	—	2	2	1	—	—	—	—	—
3	—	1	10	29	32	32	35	31	28	25	13	3	—	—	—
4	—	11	73	81	78	79	85	79	72	55	32	10	2	—	—
5	—	11	73	81	87	89	89	89	88	80	63	21	9	1	—
							"Maincrop"								
2	—	—	—	—	—	—	1	—	1	1	—	—	—	—	—
3	—	—	3	4	2	1	4	4	4	9	4	2	—	—	—
4	—	—	4	5	9	6	10	11	9	13	8	4	—	—	—
5	—	—	4	12	38	26	29	22	20	23	19	9	1	—	—

(*b*) ***Histological observations.***

Penetration. Previous workers have been agreed that penetration is exclusively stomatal, and this observation is confirmed. The spores appear

equally capable of germinating in water or in moist air on the surface of the leaf. The germ tubes are slender, about 2–3μ. in diameter, and penetrate the stomatal aperture directly or by means of a lateral branch. No appressoria or other modifications are formed. Immediately after penetration the diameter of the hyphae appears considerably increased. The orientation of the germ tubes on the surface of the leaf is apparently haphazard, although penetration was actually observed rather more frequently in the neighbourhood of the smaller vascular bundles. Frequently the germ tubes were observed to pass across the stomatal aperture without penetration. On the other hand, penetration was only rarely prevented by the presumed closure of the stomata in darkness. Possibly the stomata retain a certain rhythm causing them to open in darkness, even if slightly and for a limited period, or perhaps closure is normally incomplete. Owing to the fineness of the hyphae, a very small residual aperture would be sufficient for penetration to occur.

Experiments were undertaken in an attempt to provide an explanation of the mechanism of stomatal penetration, and the effects of varietal differences and of differences in age or position of the leaf were investigated at the same time. The experiments were carried out in a controlled environment chamber (Wilson, 1937), the air in which was maintained at a temperature of 22·5° C. and at 85 % relative humidity. Each variety was represented by leaves taken from two different regions of the plant. The leaves were numbered similarly to those in the experiment recorded in Table I, and were at a similar stage of development. The three apical leaflets were inoculated in each case. These latter were placed in Petri dishes containing moistened filter paper and were inoculated (on the lower surface) with a standard spore suspension containing the spores from a 2-month colony on quaker-oat agar to 5 c.c. of sterile water. Each experiment was in two series; in one the lids of the dishes were left in position, giving constant saturation, while in the other the lids were removed during the day and replaced at night, giving a fluctuation between saturation and 85 %, the humidity of the air in the chamber. The filter paper in the dishes was moistened when necessary. After 5 days, the numbers of penetrating hyphae were recorded over equal areas removed with the aid of a cork borer 1·4 cm. in diameter and examined by the lacto-phenol method. The experiments are summarized in Table II, the figures showing that, in a total of over six hundred instances, penetration was roughly 7·5 times more frequent in the "fluctuating" series than at constant saturation. That this was not due merely to the evaporation of the water droplets and the consequent

removal of the mechanical barrier created by their surface tension, in the former series, was shown by the fact that a similar result was obtained in an experiment in which both series of dishes, before transferring to the chamber, were left uncovered on the bench until all traces of moisture on the surface of the leaf had evaporated. It is noticeable that the results of Exp. XLIII, in which the chamber was maintained in darkness, are in no way unusual. The evidence as a whole points to the conclusion that penetration is controlled largely by a stimulus depending on evaporation from the leaf. Least difficulty is involved in the assumption that the stimulus is actually hydrotropic, and further circumstantial evidence in support of this is provided in the following sections. In Exps. XLV and XLVI, the results were analysed for differences between varieties and between leaf ages respectively (Table III). In each case, the frequency of penetration was considerably lower in "Maincrop" than in "Giant Red". As regards the different leaf ages, the results of the second experiment only were significant (i.e. to the 5% level of probability), penetration being less frequent in the upper position. This might account for the

Table II

Summary of measurements of the frequency of penetration in relation to humidity

Exp.	Variety	Position of leaf	No. of leaflets examined	Nos. of hyphae penetrating in equal areas under different conditions: "Constant"	"Fluctuating"
XLI	GR, M	VII only	12	39	137
XLII	GR only	Do.	6	—	18
XLIII	GR, M	Do.	12	21	85
XLV	GR, M	III, VII	24	4	175
XLVI	GR, M	VI, XI	24	8	114
XLVII	GR only	VII only	6	1	21
Totals:			84	73	550
Averages for each series:				1·7	13·1

GR and M respectively represent the varieties "Giant Red" and "Maincrop".

Table III

Summary of measurements of the frequency of penetration in different varieties and in different regions of the plant

(Numbers of hyphae penetrating in equal areas)

	"Giant Red"			"Maincrop"		
Exp.	Lower	Middle	Upper	Lower	Middle	Upper
XLV	92	46	—	17	24	—
XLVI	—	54	18	—	35	15
		210			91	

extreme localization of infection frequently seen in the upper leaves (see Pl. XI, fig. 2). In the uppermost leaves, corresponding to Nos. XIV–XV in these experiments, penetration was rarely observed, but this may be accounted for by the fact that at this stage the stomata are extremely small and undeveloped, the majority being apparently non-functional.

Initial development of mycelium. The penetrating hyphae produce an extensive intercellular mycelium, from which no haustoria are developed. Growth is characterized by the production of long, straight "runners", passing between the cells of the spongy mesophyll and sending out further ascending branches at intervals (Text-figs. 1, 2). The hyphae are approximately 4–5 μ. in diameter and are typically septate, densely granular and non-vacuolate. They show an increasing tendency to become localized in the vascular region (Text-fig. 4) but were not actually observed in contact with the tracheids, since they are apparently unable to penetrate between the cells of the surrounding parenchyma (Pl. XI, fig. 4). Measurements illustrating the development of an internal mycelium in susceptible and resistant varieties are summarized in Table IV. Comparison of the rate of spread of mycelium under different conditions is complicated by the steady increase in the total number of penetrating hyphae observed on successive days. Nevertheless, the results expressed in Table V indicate a strong tendency for the rate of spread to be greater in "Giant Red" than in "Maincrop". The measurements in Table V were made at random over equal areas of the leaf in each experiment. A difference of more than three times the standard deviation is held to indicate significance. According to the analysis of the results of Exp. XLV, the rate of penetration is also significantly lower in the lower leaves (No. III) than in the middle of the plant (No. VII). Detailed measurements for the upper leaves in Exp. XLVI were not available owing to the reduced frequency of penetration in this position (p. 284).

Table IV

Initial development of mycelium in susceptible and resistant varieties. (Equal areas examined)

	"Giant Red"		"Maincrop"		
Days	No.	Av. diam. μ.	No.	Av. diam. μ.	Total no. of penetrating hyphae
1	1	15	—	—	1
2	5	47	4	26	9
3	17	44	11	51	28
6	45	751	46	653	91

The diameter is that of the minimum circle within which all the hyphae from any one stoma may be included.

Table V

Comparative rate of spread of mycelium

Exp.	Days from inocu-lation	Total no. of measure-ments	Position of leaf	Av. diam. of mycelium μ. GR	M	Standard deviation
XI	6	50	VII	763	532	68
XLV	5	32	III	458	142	23
		32	VII	653	444	91*
XLVI	5	48	VI	305	165	40

* The difference is less than three times the standard deviation.

Sporulation. Under favourable conditions, sporulation commences after 10–11 days. The conidiophores originate from stromatic bodies formed in the substomatal cavities and they emerge through the greatly enlarged stomatal aperture (Pl. XI, fig. 5). If sporulation is prevented or delayed by unfavourable conditions, the stromata remain sterile while increasing in size. Eventually, the epidermis may be ruptured and occasionally a few short aerial hyphae are produced (Pl. XII, fig. 1). During this period, the hyphae themselves increase in diameter and become vacuolated. The mycelium as a whole becomes very much more abundant and stromatic aggregations develop also around the vascular bundles, many of which become completely enveloped in a sheath of hyphae.

Final stages. Yellowing of the foliage in the later stages of infection is commonly associated with the occurrence of necrosis affecting groups of cells, particularly in the palisade region. The condition is especially evident at the centre of the localized lesions on "Maincrop", and it appears to result from a withdrawal of water by the abundant development of the mycelium. The associated hyphae are swollen and highly vacuolated. Later, when the supply of food materials in the leaf is seriously diminished, the hyphae tend to become lobed and distorted (Pl. XII, fig. 2). In the final stage, after the death of the cells, the walls are broken down and the mycelium consequently appears intracellular. Intra- as well as intercellular mycelium was described by Makemson (1918) and by Chamberlain (1932), but according to the present observations the fungus should be regarded as strictly intercellular. *Cladosporium fulvum* appears to flourish in the leaf only so long as the tissues are alive; after the death of the cells, only a limited and in some ways abnormal development can occur.

(2) *Experiments with other* Lycopersicum *species*

No symptoms were recorded on any of the small-fruited tomatoes inoculated with the exception of *Lycopersicum Humboldtii* Dun., from Copenhagen, and the two strains received as *L. racemigerum*, from the John Innes Horticultural Institution. These had previously been reported immune (Bond, 1936). In several respects, they appeared closer to the cherry tomato, *L. cerasiforme* Dun., than to the true currant tomato. The latter was reported immune to *Cladosporium fulvum* by Sengsbusch & Loschakowa-Hasenbusch (1932), Osmun (1934), Alexander (1934), Guba (1936), Langford (1937), and previously by the writer (1936). The species is variously described as *Solanum racemigerum* (Sansome, 1933) or as *Lycopersicum racemigerum* Lange (= *L. racemiforme* Lange), also as *L. pimpinellifolium* Dun. (Bailey, 1922; Lindstrom, 1932) and *L. pimpinellifolium* Mill. (Guba, 1936). The latter is preferred on grounds of priority. The genetic basis of immunity in the currant tomato has recently been elucidated by Langford (1937), whose work confirms and extends that of previous investigators. In addition to the factor controlling immunity, a second factor occurs which confers a degree of partial resistance. This latter, however, is not identical with the factor for resistance in the varieties of *L. esculentum*. Schmidt's (1933) theory of the nature of immunity in the currant tomato is unfortunately untenable, having been founded on a misconception as to the identity of the causal organism (Raabe & Sengsbusch, 1935).

Histological observations. Langford (1937) described the occurrence of stomatal penetration in the currant tomato, and this observation is confirmed. Penetration occurs in the same manner and apparently with the same readiness as in the commercial varieties. Moreover, the effect of humidity on the frequency of penetration was also similar, totals respectively of 68 and 189 penetrating hyphae being recorded for the "constant" and "fluctuating" series in Exps. XLI–XLIII, in which the "S.F. 3" currant tomato was inoculated together with the varieties "Giant Red" and "Maincrop" (see p. 283). The effect was equally noticeable in leaflets inoculated and maintained in darkness. The mycelium after penetration is intercellular and without haustoria. Its extent appears to depend on the relative maturity of the leaf, which in turn is influenced by cultural conditions and the season of the year. In immature leaves and in young leaves developed during the winter months (thin, "shade" leaves with abundant intercellular spaces), it rapidly becomes stoloniferous to a degree comparable with that observed

in the early stages on "Giant Red" tomato. The host cells appear unaffected by the presence of the hyphae. Beyond this stage, however, no further development occurs. A gradual increase in diameter may take place, but the long runners remain comparatively scattered and far apart, not giving rise to any stromatic aggregations (Text-figs. 3, 4). The maximum length of time during which the mycelium remains alive under these conditions was not determined. The development of an extensive, stoloniferous mycelium takes place only under the conditions described. As the leaves become older, and in leaves developed under progressively better conditions, the mycelium is more and more restricted. At the same time there is a tendency for the host cells in immediate contact with the hyphae to become necrotic. In general, only isolated cells were affected and no external symptoms were produced. Langford (1937), however, observed a flecking of the foliage after inoculation in five only out of more than a hundred experiments. In the final stage, the mycelium is greatly restricted, less than 100μ. in diameter, and in this condition frequently becomes excessively branched (Text-fig. 5). Measurements illustrating this course of events are summarized in Table VI. The figures are intended to be representative only; the records always showed great variability and no statistical analysis was attempted.

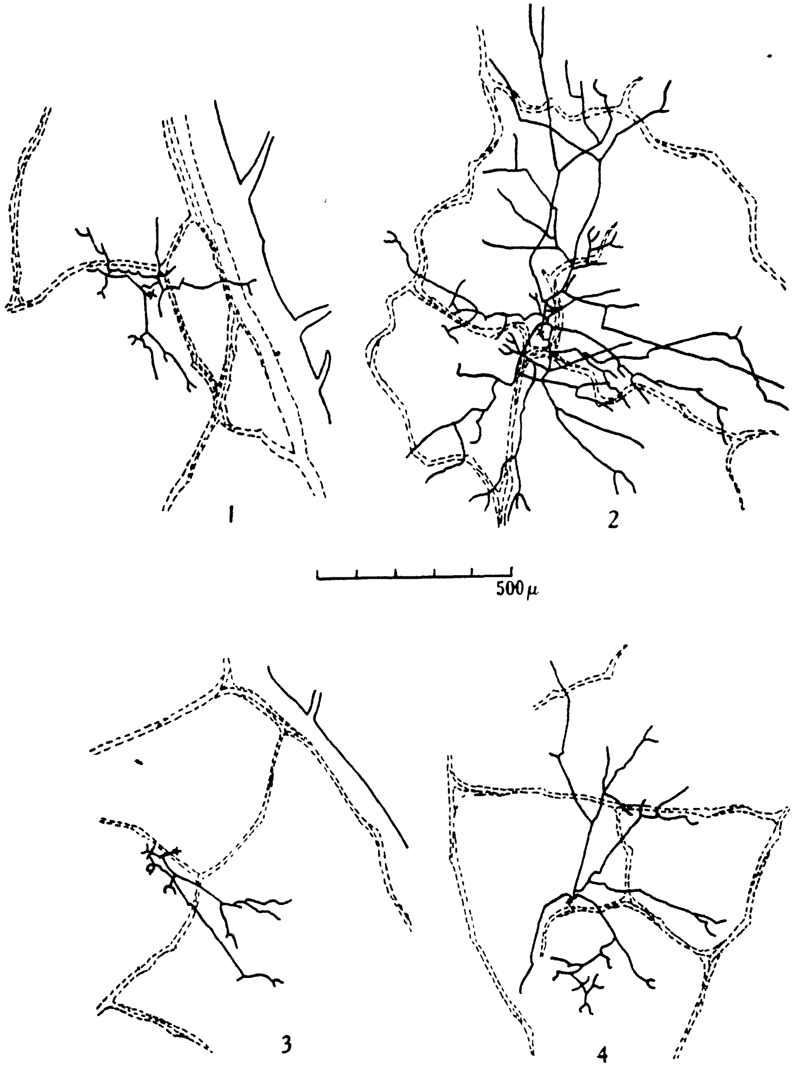
Table VI

Rate of spread of mycelium in foliage of currant tomato at successive stages of development

Stage	Description	Days from inoculation	No. of measurements	Mycelium Av. diam. μ	Mycelium Maximum μ.
A	First and second leaves of seedlings, in February	4	14	400	640
			18	350	760
		6	3	215	340
			6	439	735
		10	2	435	640
		17	7	320	860
B	Young leaves, March–April	1	1	10	10
		2	2	33	41
		3	3	75	150
		6	8	180	300
			7	150	225
C	Mature leaves, May–June	6	6	58	90

(3) *Experiments with immune and "inappropriate" hosts*

No external symptoms of infection were recorded on any of the plants inoculated and all, by this criterion, were entirely immune. Similar failures to extend the host range of the fungus were reported by Sengsbusch & Loschakowa-Hasenbusch (1932), Guba (1936), and other



Text-figs. 1–4. Course of penetrating hyphae. (These and subsequent text-figures are from lacto-phenol preparations, viewed from the lower surface of the leaf, and drawn with the aid of a camera lucida.)

Text-fig. 1. "Giant Red" tomato, after 4 days.

Text-fig. 2. "Giant Red" tomato, after 7 days.

Text-fig. 3. "S.F. 3" currant tomato, after 6 days (stage "A").

Text-fig. 4. "S.F. 3" currant tomato, after 17 days (do.).

workers. *Cladosporium fulvum* was recorded as a pathogen of *Solanum melongena* by Ciferri (1927), in the Dominican republic, but the same author later (1930) stated that the disease was seldom seen and was not clearly identified. Pritchard & Porte (1921) also noted a closely similar fungus infecting *S. carolinense* in the eastern United States, but were again unable to confirm its identity by inoculation experiments.

Histological observations. Examination of inoculated, fixed material of the apparently immune plants revealed the ready occurrence of stomatal penetration in all species with the exception of 10-week stock (*Matthiola* sp.), in which the epidermis on both surfaces of the leaf is protected by a dense covering of stout, branched hairs. On the other plants the penetrating hyphae pass directly through the stomatal aperture in the manner previously described for *Lycopersicum esculentum*. Penetration is not normally prevented by the presumed closure of the stomata in darkness. Throughout the experiments as a whole, penetration appeared less frequent than in susceptible hosts. Thus, in a whole series of inoculations carried out under uniform conditions and involving approximately equal areas of foliage, the average frequency of penetration per sq. cm. was 14·5 for three tomato varieties and 7·1 for ten other solanaceous plants. In some instances, however, this relationship was appreciably modified, or even reversed. In each of two experiments involving both *Nicotiana tabacum* and *Antirrhinum majus*, the frequency of penetration was higher at fluctuating humidity than at constant saturation, the difference being significant at the 5 % level of probability (see p. 283). This suggests a mechanism of penetration similar to that which is effective in susceptible hosts. The internal mycelium is uniformly intercellular and without haustoria but it varies greatly in extent in the different species and under different conditions. Wherever possible the diameter of penetration was recorded 1, 2, 3, and 6 days after inoculation and again after an interval of about 4 weeks. In some plants the series of observations was less complete. The results as a whole seemed to fall into three groups, which are discussed separately.

The first group of results (Table VII) comprises eight different solanaceous plants in which the maximum diameter of penetration after 6 days was 200μ. or greater. As in the currant tomato, the extent of the mycelium varies with the age or condition of the foliage, and this is illustrated by the grouping of the results in three columns "A", "B", and "C", corresponding to the three stages recognized in Table VI. In some plants, for instance *Solanum melongena*, these represented fairly well-marked morphological stages in the development of the leaf, but in

no case could any absolute distinction be drawn. The maximum diameter of penetration is associated with young foliage, developed early in the year and under reduced light intensity, thin, and with abundant intercellular spaces. In this condition the mycelium is frequently stoloniferous, often with subepidermal hyphae following the grooves marking the inner edge of the radial walls of the epidermal cells (Text-fig. 6). The host cells appear but little affected by the parasite, and such response as was noticed (chiefly discoloration and presumed eventual necrosis) was comparatively slight and infrequent. Similarly, the fungus mycelium is normal in appearance and is checked only in the vigour of its development, suggesting a deficiency of some necessary food material. As the cells of the leaf become better developed, the diameter of penetration is reduced and necrosis appears frequently, probably on account of the greater closeness of contact between the host cells and the invading hyphae. Finally, the mycelium is restricted to one or two short branches between the cells immediately adjacent to the stomatal cavity (Text-figs. 7, 8) or even to a few swollen, peg-like outgrowths actually in contact with the inner face of the guard cells. If this condition is associated with necrosis, it is sometimes impossible to determine whether the hyphae have actually passed the stomatal aperture or not.

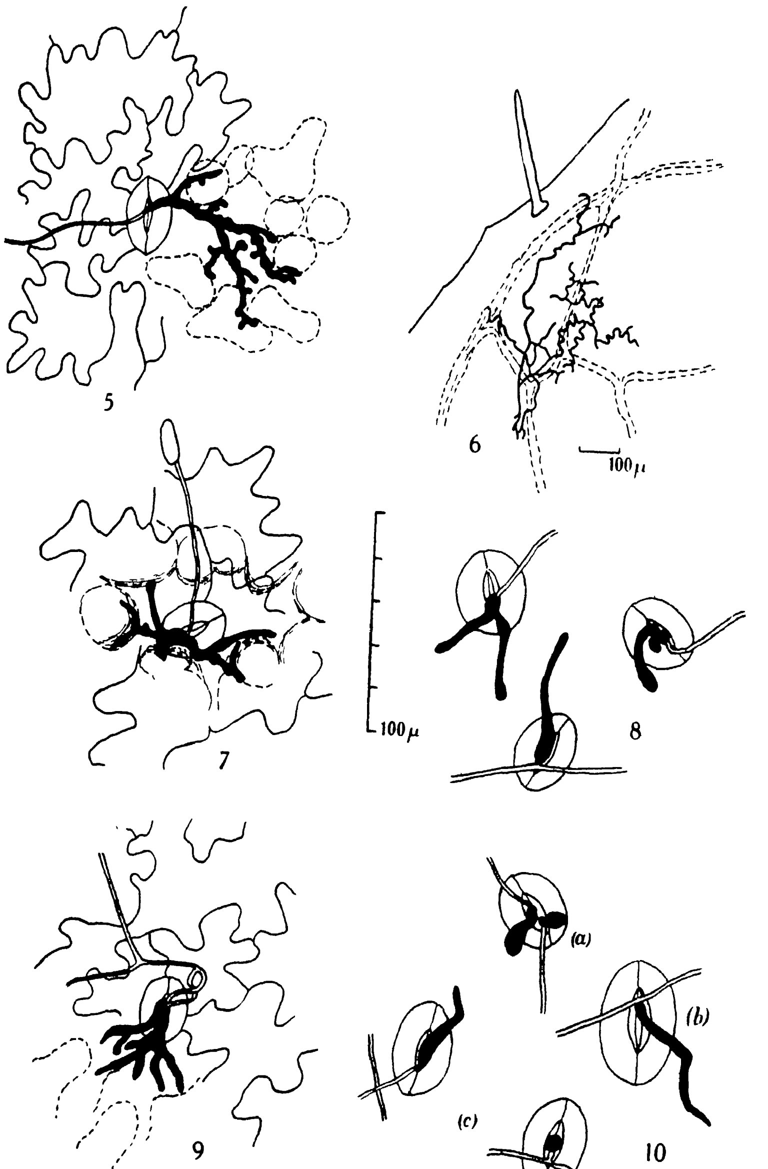
Table VII

Rate of spread of mycelium in eight other solanaceous hosts
(Number and extent of penetrating hyphae)

	"A"			"B"			"C"		
Days from inoculation	No.	Av. diam. μ.	Max.	No.	Av. diam. μ.	Max.	No.	Av. diam. μ.	Max.
1	2	25	25	--	—	—	—	—	—
2	18	38	110	—	—	—	—	—	—
3	69	85	450	15	39	125	—	—	—
6	103	215	1020	66	50	90	12	32	70
25–29	1	650	—	91	69	300	107	36	90

The following species are represented: *Solanum melongena, S. dulcamara, Schizanthus grahamii, Atropa belladonna, Hyoscyamus niger, Nicotiana tabacum, N. sylvestris, N. glutinosa.*

The second group (Table VIII) includes species in which the maximum diameter of penetration was more than 100μ., but did not exceed 200μ. after the first 6 days. Leaves in the juvenile or "A" condition were apparently comparable in every respect with those of species previously considered, in which the development of mycelium was usually far more extensive. The diameters attained, however, were more comparable with



Text-figs. 5–10.

those recorded for the "B" condition in the previous table. Consequently, the figures are considered in two groups only, "A" and "B" (Text-fig. 9).

Table VIII

Rate of spread of mycelium in four other solanaceous hosts
(Number and extent of penetrating hyphae)

	"A"			"B"		
Days from inoculation	No.	Av. diam. μ.	Max.	No.	Av. diam μ.	Max.
2	3	27	35	—	—	—
3	27	35	75	—	—	—
6	89	53	145	12	17	30
25–29	5	250	300	73	19	85

The following species are represented: *Solanum capsicastrum, S. nigrum, Datura stramonium, Browallia viscosa.*

In the remaining species, including the Capsicums and the "inappropriate" hosts belonging to other families, penetration was even more reduced. The maximum diameter after 6 days was that of 110 μ. in *Callistephus* sp. In *Antirrhinum majus* and *Bryonia dioica*, however, penetration was not observed to exceed 50 μ. even in the youngest leaves, the mycelium being confined typically to single, peg-like or slightly swollen branches (Text-fig. 10). In contrast to the species included in the two previous groups this restriction in the development of mycelium is rarely associated with necrosis, thus indicating the possible existence of a factor initially unfavourable to the fungus, not depending on an active reaction between the host cells and the invading hyphae.

IV. *CLADOSPORIUM CUCUMERINUM*

Cladosporium cucumerinum Ell. & Arth. is known in this country as the cause of cucumber "gummosis", which is a disease largely confined to the fruits. In the United States it is also known to cause a

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Text-fig. 5. Limited mycelium in "S.F. 3" currant tomato, after 6 days (stage "C": mature).

Text-fig. 6. Extensive mycelium in *Solanum melongena*, after 18 days, showing subepidermal hyphae in centre (stage "A").

Text-fig. 7. Limited mycelium in *Hyoscyamus niger*, after 6 days, showing ring of discoloured cells (stage "C").

Text-fig. 8. Short hyphae in *Solanum melongena*, after 29 days, not associated with discoloured cells (stage "C").

Text-fig. 9. Limited mycelium in *Solanum nigrum*, after 6 days (stage "A").

Text-fig. 10. Penetration in "inappropriate" hosts, after 6 days, not associated with discoloration.

(*a*) *Antirrhinum majus*, (*b*) *Bryonia dioica*, (*c*) *Callistephus* sp.

destructive disease of the foliage and seedling plants, referred to originally as "leaf blight" (Humphrey, 1892) or more recently as "scab" (Doolittle, 1916). A similar condition has been reported from Holland (Pfältzen, 1927; Muyzenberg, 1932) and other countries (Eriksson, 1915). According to v. Höhnel (1923), the fungus *Scolecotrichum melophthorum* Prill. & Del., causing "La Nuile" of melons (Prillieux & Delacroix, 1891) and cucumbers (Delacroix, 1892) in France is identical with *Cladosporium cucumerinum*. This synonymy was upheld by Garbowski (1924) and was later confirmed by the inoculation experiments of Pfältzen (1927). In England the fungus appears to have been described first by Cooke (1903, 1903*a*), who named it *C. scabies* Cke. Both strains of *C. cucumerinum* investigated produced extensive fertile colonies on both Dox's medium and quaker-oat agar, growth being only a little slower than in *C. herbarum* (Pl. XII, fig. 3). Consequently, they were readily distinguishable in appearance from *C. fulvum*. Colonies of strain 1/36 (from Cheshunt) were generally paler in colour than those of strain 2/35 (from Baarn), and the spores were larger in all dimensions. Spore dimensions of *C. cucumerinum*, *C. fulvum*, and *C. herbarum* are summarized in Table IX.

Table IX

Spore measurements for three species of Cladosporium

		One-celled		Two-celled
C. fulvum		(89%)		(10%)
(Average of 4 strains)	(200)	$16{\cdot}2 \pm 0{\cdot}26 \times 6{\cdot}8 \pm 0{\cdot}07$	(200)	$26{\cdot}9 \pm 0{\cdot}41 \times 6{\cdot}6 \pm 0{\cdot}08$
C. cucumerinum		(79%)		(21%)
1/36	(50)	$15{\cdot}2 \pm 0{\cdot}73 \times 5{\cdot}0 \pm 0{\cdot}13$	(25)	$22{\cdot}5 \pm 1{\cdot}00 \times 5{\cdot}7 \pm 0{\cdot}17$
2/35	(50)	$11{\cdot}8 \pm 0{\cdot}44 \times 4{\cdot}1 \pm 0{\cdot}11$	(25)	$16{\cdot}4 \pm 1{\cdot}05 \times 4{\cdot}6 \pm 0{\cdot}26$
C. herbarum		(96%)		(4%)
	(50)	$4{\cdot}7 \pm 0{\cdot}18 \times 3{\cdot}5 \pm 0{\cdot}09$	(10)	$15{\cdot}0 \pm 0{\cdot}70 \times 4{\cdot}9 \pm 0{\cdot}26$

Dimensions are in μ.

Spores of *C. fulvum* were obtained from "Giant Red" tomato foliage, 17 days after inoculation.

Spores of *C. cucumerinum* and of *C. herbarum* were obtained from colonies on Dox's medium, after 3 weeks at 25° C.

(*a*) *Proof of pathogenicity*

Inoculations were carried out on seedling plants and detached leaves of Carter's "Telegraph" cucumber and of *Bryonia dioica*, also on cucumber fruits (variety unknown) and on plants and leaves of the following "inappropriate" hosts, namely, "Giant Red" and "Maincrop" tomato, the "S.F. 3" currant tomato, *Solanum nigrum*, *Antirrhinum majus*, *Callistephus* sp. Both strains were pathogenic to cucumber fruits. Needle inoculation with spores removed from a colony on Dox's medium

produced sunken lesions attaining a diameter of about 1·5 cm. after the first 8 days (Pl. XI, fig. 4). Sporulation was evident after the second day, the lesions being similar in colour to the colonies on Dox's medium. Frequently they were surrounded by a yellowed or paler area. No trace of gum exudation was observed on these lesions, and van der Muyzenberg (1932) had previously noted a similar absence of gummosis in certain instances. Similar lesions were observed repeatedly after reisolation and subsequent inoculation, the reisolates also retaining the cultural characteristics of the parent strains. Inoculations with *Cladosporium herbarum* and with the *C. fulvum*, carried out under similar conditions, gave negative results, a very scanty growth of the fungus being observed only at the actual point of inoculation. Spore germination by *C. cucumerinum* in distilled water was unsatisfactory and for spray inoculations the spores were usually applied in half-strength Dox's solution, using the same solution for the control experiments. On cucumber seedlings infection was severe, but the symptoms were confined to the basal and apical regions of the plant and were rarely so extensive as those described by Humphrey (1892) and later by van der Muyzenberg (1932). The apex was usually destroyed, and on the lower leaves large, water-soaked lesions developed, with abundant sporulation. Small necrotic lesions occurred on the stems, petioles, and veins of the leaves; these were frequently associated with a drop of gummy exudation. From the results available no distinction could be drawn affecting the pathogenicity of the two strains investigated. Strain 2/35 was also found to cause the destruction of the young stems, leaves and tendrils of *Bryonia dioica*, but no symptoms were observed on any other plant inoculated.

(b) *Histological observations*

Cucumber fruits. In the early stages of infection, the hyphae are many-septate and irregularly swollen, about 8–10μ. in diameter. They penetrate the cell walls readily and apparently by mechanical pressure, no evidence of enzyme action being observed. The cells in advance of the hyphae and those in lateral contact with them appeared unaffected. No special aggregations of hyphae are formed in the neighbourhood of the vascular bundles. The mycelium penetrates into actual contact with the xylem elements, many of which are invaded by hyphae passing between the thickened spirals. Ultimately, the outer tissues of the rind are destroyed and the mycelium becomes consolidated to form a dense, superficial crust, 100μ. or more in thickness. Afterwards, there is probably a more limited and chiefly intercellular development in the

lower layers, in which the cells are larger and have thicker walls more able to resist penetration (Pl. XII, fig. 5).

Cucumber foliage. Treatment with chloral hydrate was necessary to ensure adequate clearing (p. 280). Penetration is stomatal, the process being similar to that previously described for *Cladosporium fulvum.* The resultant mycelium is initially intercellular, without haustoria. The hyphae early become swollen, reaching a breadth of 8–10μ., but they are not distorted as they are in the fruits. The growth of the mycelium is sometimes very rapid, a maximum extent of 350μ. having been recorded 3 days after inoculation. Branching is characteristically dendritic (Text-fig. 11). The long, straight runners typical of the mycelium of *C. fulvum* on tomato are not formed and there is no tendency to the formation of stromatic aggregations enclosing the vascular bundles. The exact period at which death of the host cells occurs was not determined. In some leaves the growth of the mycelium is arrested before any effect on the host is noticeable. Occasional sections, cut 10 days after inoculation, showed the mycelium still apparently intercellular, with single conidiophores emerging through the stomata. The more normal condition is for sporulation to occur only in those parts of the leaf previously killed by the parasite, when the mycelium is apparently intracellular, with the conidiophores passing directly through the remains of the epidermal walls.

"Inappropriate" hosts. Normal stomatal penetration was observed repeatedly on all other plants inoculated, with the following maximum diameters of penetration 6 days after inoculation: "Giant Red" tomato 50μ., "Maincrop" tomato 45μ., "S.F. 3" currant tomato 85μ., *Solanum nigrum* 10μ., *Antirrhinum majus* 80μ. (Text-fig. 12). Penetration is not usually associated with necrosis or discoloration of the cells. The hyphae are frequently unbranched, ascending rapidly towards the palisade; subsequent measurements failed to reveal any significant increase in

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Text-fig. 11. *C. cucumerinum* in foliage of *Cucumis sativus*, after 8 days.

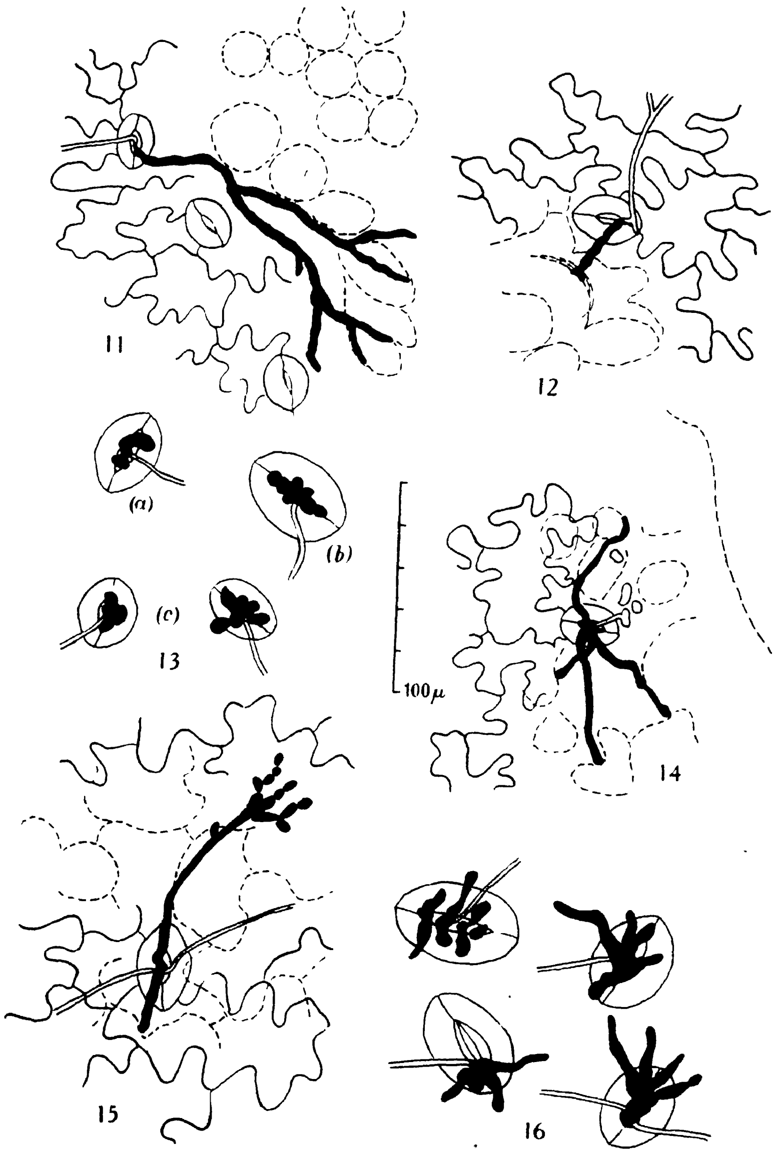
Text-fig. 12. *C. cucumerinum* in *Antirrhinum majus*, after 6 days.

Text-fig. 13. *C. cucumerinum* in *Antirrhinum majus*, "peg"-like outgrowths, after 25 days, in older foliage. (*a*) *Lycopersicum esculentum*, (*b*) *Solanum nigrum*, (*c*) *Antirrhinum majus*.

Text-fig. 14. *C. herbarum*, extensive mycelium in currant tomato, after 6 days, young condition.

Text-fig. 15. *C. herbarum* in *Solanum nigrum*, young condition, showing internal sporulation.

Text-fig. 16. *C. herbarum* in *Solanum nigrum*, limited mycelium in mature foliage, associated with necrosis.



Text-figs. 11–16. *C. cucumerinum* and *C. herbarum*.

extent after the 6-day period. In older leaves the mycelium rarely develops beyond one or more swollen, peg-like outgrowths, similar to those produced under comparable conditions by *Cladosporium fulvum*. These appeared unchanged after an interval of 25 days from inoculation (Text-fig. 13).

Frequency of penetration. The effect of humidity on the frequency of penetration was investigated by the method described for *C. fulvum* (p. 283). In each of two experiments, involving "Telegraph" cucumber, "Giant Red" tomato, *Solanum nigrum*, and *Antirrhinum majus*, the frequency of penetration was higher at "fluctuating" humidity than at constant saturation, but the difference failed to reach significance at the 5 % level of probability. No conclusions as to the mechanism controlling penetration can be drawn from these experiments. Considering the relatively high concentration of spores in the suspensions used for inoculation, and their abundant germination, the frequency of penetration at either condition of humidity was always surprisingly low. The possibility remains that such penetration as there is occurs entirely by chance, although the influence of such factors as the age of the spores and the conditions of germination must not be overlooked. Limited observations with spores germinated in distilled water indicated that the use of a nutrient solution to ensure adequate germination had no effect on the subsequent course of events described above.

V. *Cladosporium herbarum*

C. herbarum (Lk.) Fr. is well known as a ubiquitous saprophyte (Brooks & Hansford, 1923; Fraser, 1934), although from time to time various parasitic activities have been attributed to it. These reports were summarized by Bennett (1928), who showed that the fungus should be regarded as a saprophyte capable of becoming "semi-parasitic" only on moribund tissues. A similar conclusion was reached later by Bockmann (1933). No evidence of pathogenicity was observed by the present writer. Inoculations of seedling plants and detached leaves of "Giant Red" and "Maincrop" tomato, "S.F. 3" currant tomato, *Solanum nigrum*, and *Antirrhinum majus* gave negative results, even when the spores were applied in a nutrient solution, to ensure adequate germination.

Histological observations. Stomatal penetration was observed in all plants inoculated, the germ tubes passing through the stomatal aperture in the manner described for the other two *Cladosporium* species. A limited mycelium is produced, which is intercellular and without haus-

toria. The mycelium differs considerably in appearance and extent according to the species and the age of the foliage inoculated. In the tomato varieties, it is confined to one or several peg-like outgrowths not more than 75μ. in extent, usually without any evident reaction of the host cells. A similar appearance was observed in the currant tomato, in which plant, however, the mycelium on one occasion reached an extent of 125μ. without necrosis (Text-fig. 14). In *Solanum nigrum*, the mycelium in young, poorly developed foliage consists typically of a single, unbranched hypha and occasionally, internal condidiophores are developed (Text-fig. 15). These were not observed on any other species. In older foliage of *S. nigrum*, penetration is usually associated with necrosis, the limited mycelium being swollen and distorted (Text-fig. 16). Possibly, this condition results in part from the confined dimensions of the stomatal cavity, which may become partly "occluded" by the growth of the surrounding mesophyll cells. Only a limited development of mycelium was recorded in *Antirrhinum majus*. In a single experiment involving all three host species, the frequency of penetration was slightly higher at constant saturation than at "fluctuating" humidity (see p. 283), but the difference was not significant to the 5% level of probability. In general, the frequency of penetration was of the same order as that observed in *Cladosporium cucumerinum*, i.e. considerably below that of *C. fulvum* under similar conditions.

VI. Discussion

Brown (1936, p. 265) has recently expressed the opinion that "the relation of the mycelium of the parasite to the host cell gives one of the best criteria for distinguishing the two types...", i.e. of obligate or facultative parasitism. The same author also refers to *Cladosporium fulvum* and some other fungi as representing the class of "facultative saprophytes" rather than "facultative parasites". These statements form a convenient basis for a discussion of the observations contained in the present communication.

The mycelium of *C. fulvum* is strictly intercellular. There are no haustoria, which are characteristic of the obligate type of parasite, and there is no evidence for any enzyme action enabling it to penetrate between host cells which are normally in contact. Apparently, the host cells may later be separated to a certain extent by the continued growth and aggregation of the hyphae in the original intercellular spaces. The growth of the fungus appears to depend on the maintenance of a sufficiently close contact between host and parasite. This is seen particularly

in the sheath of hyphae surrounding the vascular bundles and, in the earlier stages, in the short, clasping hyphae attached to the free ends of the palisade cells. As to how far this contact may be compared with the union effected by a haustorial parasite remains uncertain. The appearance suggests an active withdrawal of water and dissolved food substances from the host cells rather than the mere utilization of the products of normal exosmosis. The absorption of water would presumably depend on a simple osmotic relationship, but the passage of dissolved substances would require an additional mechanism involving possibly an active influence exerted by the fungus on the permeability of the protoplasm of the host cells. Death of the host cells is accompanied by abnormalities in the associated hyphae which become progressively swollen and distorted and make no further active development. In this respect a strong resemblance is shown with the obligate type of parasite. However, there is no obvious mechanism of resistance comparable with the "hypersensitive" reaction to certain rust fungi. On the other hand, even in the earliest stages of infection the rate of spread of mycelium is less in the resistant varieties, just as it is in the relatively resistant lower leaves of an individual plant. That the factor for varietal resistance is a relatively simple one is suggested by its inheritance as a single Mendelian character (Guba, 1936). However, the existence of so many degrees of resistance among the different varieties suggests that a quantitative reaction may be involved. The behaviour of Langford's (1937) recently described physiologic race, which is reported to produce "susceptible" symptoms on varieties resistant to the normal strains, seems to lend further support to the view that resistance results from some relatively simple character. Langford's mutant strains, producing necrotic non-sporing lesions on resistant and susceptible types alike, recall the sterile variants originally described by Caldis & Coons (1927). Similar variant strains have been isolated by the present writer. The peculiar type of lesion produced appears to result from the initially more rapid development of the sterile strains (as compared with the fertile types), leading to their own arrest through the premature exhaustion and death of the host cells. The mechanism of stomatal penetration by *C. fulvum* on tomato was discussed in connexion with the experiments described on p. 283. The writer agrees with Brown (1936, p. 245) that "no difficulty is involved in assuming that a germ tube grows accidentally over one stoma or another". The experiments indicate that "the second stage of the process" (*loc. cit.*), that is the actual initiation of penetration by the apex of the germ tube or by a newly developed lateral branch, is controlled in

part by a stimulus depending on the evaporation of water from the leaf. The fact that similar results were obtained with immune and inappropriate hosts suggests that the stimulus to penetration is hydrotropic, i.e. entirely non-specific to the susceptible hosts. The experiments recorded are believed to be the first in which evidence as to the mechanism of stomatal penetration has been obtained from the use of controlled conditions of humidity affecting the frequency of penetration as it occurs on the host plant. The only previous work of significance on this point, by Balls (1905), involved the use of artificial membranes of perforated rubber.

In view of the above conclusion the problem of the invasion of immune and inappropriate hosts is primarily that of accounting for the loss of pathogenicity and of explaining the differences in extent and distribution of the mycelium in the different species. Similar questions were raised by the experiments of Gibson (1904), Young (1926), Johnson (1932), Corner (1935), Hanes (1936), and other workers, although the organisms investigated differed from *C. fulvum* either by the formation of appressoria for stomatal or direct penetration or by the haustorial habit of their mycelium. In the currant tomato the distribution of the internal mycelium of *C. fulvum* depends largely on the age and structure of the particular leaf inoculated. The extensive mycelium produced in the young, poorly developed leaves is apparently parasitic to a certain extent. Possibly, immunity at this stage will prove closely similar to resistance in "Maincrop" tomato, the two types of reaction differing only in degree. In the adult condition this difference might become more pronounced, but the frequent occurrence of an active necrotic reaction of the host cells would appear to represent an additional mechanism of a different type. Indirect evidence of this may possibly be deduced from the existence of two independent factors controlling resistance and immunity respectively in Langford's (1937) *pimpinellifolium* × *esculentum* crosses. In at least eight other solanaceous species, from five different genera (Table VII), the course of events is essentially the same as in the currant tomato. In other species (Table VIII) a somewhat reduced diameter of penetration can be correlated with certain structural or histological peculiarities. The case of *Solanum nigrum* has already been mentioned (p. 299). *Browallia viscosa*, again, appears to have unusually small cells with poorly developed intercellular spaces. Finally, in the Capsicums and in the plants belonging to other families, the mycelium remains restricted even in the young foliage, although usually without any necrotic reaction of the host cells. The observations recorded admit

the possibility that immunity in the currant tomato and in many other Solanaceae may be genetically related, while in the last group of plants mentioned there would appear to exist genuine and entirely unrelated "non-susceptibility" to *Cladosporium fulvum* (Reddick, 1928), the nature of which remains unsolved.

C. cucumerinum and *C. herbarum* agree closely with *C. fulvum* in their manner of stomatal penetration and in the habit of the resultant mycelium. Where the mycelium of *C. cucumerinum* is intracellular, in the thin-walled rind tissues of the cucumber, the hyphae appear to penetrate the cell walls by mechanical pressure. In the thicker walled inner layers they are more frequently confined to the intercellular spaces. The behaviour of *C. cucumerinum* on cucumber foliage differs from that of *C. fulvum* on tomato chiefly in the fact that sporulation is largely delayed until the host cells are dead. This recalls its more ready growth on artificial media and indicates that it should be classified as a true facultative parasite. The spores are also smaller than those of *C. fulvum* and are less able to germinate in the absence of nutrients. On "inappropriate" hosts, the mycelium is not usually associated with necrosis of the host cells. An interesting feature is the possibility of the existence of distinct strains showing different pathogenic activities, particularly as regards their virulence to the foliage and seedling plants. The behaviour of *C. herbarum* in the present investigation confirms the generally accepted opinion of Bennett (1928) and other workers, that it is essentially saprophytic. On all plants inoculated the mycelium remains limited in extent and gives no indication of parasitic activity.

The question arises as to how far the pathogenic activity of the three species investigated will prove to be characteristic for the genus as a whole. The most important features under discussion can be summarized as follows: (1) stomatal penetration by unaltered germ tubes, (2) mycelium in the foliage typically intercellular, not producing haustoria, (3) conidiophores essentially subepidermal in origin, not forming superficial or subcuticular layers. In one species at least, namely, *C. paeoniae* Passer, this behaviour is probably closely paralleled. According to Meuli's (1937) recent account the mycelium of this species is at first largely superficial, with occasional stomatal penetration. Eventually, after the death of the host tissues (which occurs in a manner still unexplained) an abundant internal mycelium is observed, with conidiophores emerging through the stomata. The third character mentioned, namely, the mode of origin of the conidiophores, may possibly be useful in determining the true systematic position of the two species *C. carpo-*

philum Thüm and *C. effusum* (Wint.) Demaree, causing "scab" of stone fruit (*Prunus* spp.) and of pecan (*Hicoria* spp.) respectively. The former species, on cherry, is now usually referred to as *Fusicladium cerasi* (Rabh.) Sacc., the perfect stage having been identified as *Venturia cerasi* Aderh. The name *Cladosporium carpophilum* is retained for the strain infecting peach and plum, in which no perfect stage has been recorded. The latter species, *C. effusum*, formerly known as *Fusicladium effusum* Wint., was transferred to the genus *Cladosporium* by Demaree (1928), on account of its catenulate conidia, a character which according to recent (unpublished) observations of d'Oliveira is of doubtful systematic value. Both these species are characterized by the formation of superficial or subcuticular stromata from which the conidiophores are developed, a feature which would tend to suggest closer affinities with *Fusicladium* Bonorden than with *Cladosporium* Lk. The same feature was also used by v. Höhnel (1923) to separate *Fusicladium* from the related genus *Passalora* Fries. A further investigation of the systematic value of these characters is desirable.

VII. Summary

Cladosporium fulvum

1. Symptoms of infection on resistant and susceptible varieties of tomato (*Lycopersicum esculentum* Mill.) are briefly indicated, together with a numerical method of recording the progress of infection.

2. The histology of infection on tomato varieties is described. Penetration is stomatal and no appressoria or other modifications are formed. The mycelium is intercellular and without haustoria, and develops normally only for so long as the host cells are alive.

3. By the use of a controlled environment chamber, the frequency of penetration is shown to be far greater at a humidity fluctuating from saturation to 85 % than at constant saturation. The suggestion is made that penetration is controlled, in part at least, by a hydrotropic stimulus.

4. The initial rate of spread of the mycelium within the host, immediately subsequent to penetration, is lower in the variety "Maincrop" than in "Giant Red" and is lower also in the basal region of the plant than in the middle. This behaviour runs parallel with the subsequent differences observed in the external symptoms of infection.

5. Normal stomatal penetration occurs over a wide range of immune and "inappropriate" hosts.

6. Symptoms of infection have been recorded only in the varieties of *L. esculentum*, in *L. Humboldtii* Dun., and in two strains received as

L. racemigerum which appear to be more closely related to the cultivated varieties than to the true currant tomato.

7. Confusion occurs as to the correct nomenclature of the currant tomato. The name *Lycopersicum pimpinellifolium* Mill. is preferred. *L. racemigerum* Lange (=*Solanum racemigerum*) and *L. racemiforme* Lange are synonyms.

8. The extent and distribution of the mycelium in the currant tomato appears to be determined largely by the age and relative maturity of the leaf. The mycelium is intercellular and without haustoria, and can apparently remain alive in the leaf for a considerable period of time. No conidiophores are produced. No reaction of the host cells is observed except where, on account of structural considerations, host and parasite are brought into more immediate contact. Here, individual host cells are frequently necrotic.

9. On many other Solanaceae, including species of *Solanum*, *Hyoscyamus*, *Nicotiana*, *Schizanthus*, and other genera, the course of events is essentially similar to that observed on the currant tomato.

10. In other Solanaceae, and in plants belonging to the Scrophulariaceae, Compositae, and Cucurbitaceae, an extensive mycelium is never developed, even under apparently favourable conditions.

Cladosporium cucumerinum and *C. herbarum*

11. *C. cucumerinum* is pathogenic to fruits and foliage of cucumber (*Cucumis sativus* L.), also to the young shoots of *Bryonia dioica* Jacq.

12. Both *C. cucumerinum* and *C. herbarum* are capable of penetrating the stomata of a wide range of plants in a manner apparently identical with that observed in *C. fulvum.*

13. *C. herbarum* is not considered potentially parasitic on the plants inoculated.

14. From a consideration of the behaviour of the three species investigated, the suggestion is made that the following features may prove characteristic of the pathogenic behaviour of the genus *Cladosporium* as a whole:

Stomatal penetration by unaltered germ tubes: intercellular mycelium without haustoria: conidiophores subepidermal in origin, not forming superficial or subcuticular layers.

Intercellular mycelium refers typically to infection of foliage. Examples of the application of this hypothesis are given.

VIII. ACKNOWLEDGEMENTS

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The writer is indebted to those workers who have supplied seed or other material or who have assisted the investigation in any way, particularly to Dr A. R. Wilson, lately of this Department, for his very generous permission to use the controlled environment chamber and for the readiness with which his experience in running it was made available.

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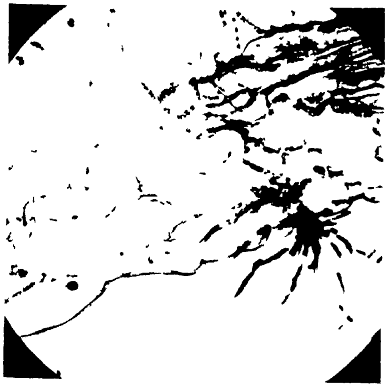
Fig 1

Fig 2

Fig 4



Fig

Fig 3

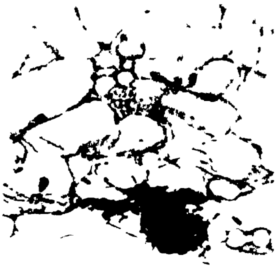


Fig 1

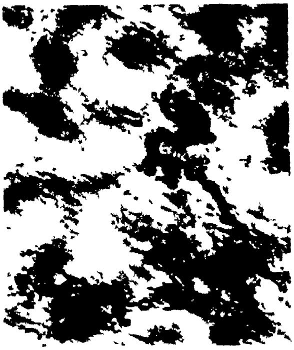


Fig 2.

a *b* *c*

Fig 3

Fig. 4.

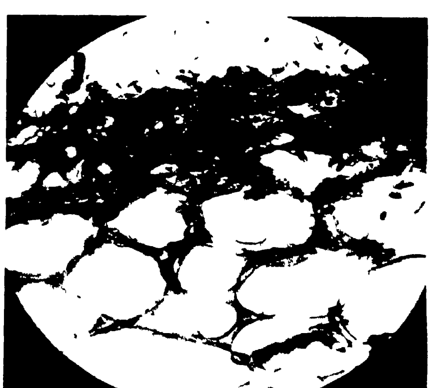


Fig. 5.

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EXPLANATION OF PLATES XI AND XII

PLATE XI

Fig. 1. Middle leaflets of "Giant Red" tomato infected 5 weeks previously. (No manure.) × ¾.

Fig. 2. Upper leaflet of "Maincrop" tomato, showing sharply localized lesions, after 5 weeks (0·5 g. sulphate of ammonia weekly). × ¾.

Fig. 3. Middle leaflets of "Maincrop" tomato, as in fig. 1. × ¾.

Fig. 4. Mycelium in "Giant Red" tomato, 4 days after inoculation. Lacto-phenol preparation, showing intercellular hypha following the vascular parenchyma. × 250. (Part of Text-fig. 1.)

Fig. 5. Sporulation in "Giant Red" tomato, showing fertile stroma with intercellular hyphae, after 12 days. Section at 12 μ., stained carbol thionin blue and orange G. × 250.

PLATE XII

Fig. 1. Sterile stroma in "Maincrop" tomato, as in Pl. XI, fig. 5. × 250.

Fig. 2. Distorted hyphae in necrotic spongy mesophyll, after 25 days. Lacto-phenol preparation. × 250.

Fig. 3. Plate colonies on Dox's medium, after 4 weeks at 22·5° C. × ⅖. (*a*) *C. cucumerinum*, strain 1/36 (smoke grey, 21′′′′′ O-YY d). (*b*) *C. cucumerinum*, strain 2/35 (citrine drab, 21′′′ O-YY i). (*c*) *C. herbarum* (dark greyish olive, 21′′′′ O-YY k). (Colours from Ridgway's *Colour Standards and Colour Nomenclature*.)

Fig. 4. Part of cucumber fruit infected with *C. cucumerinum*, strain 2/35, after 9 days at 22·5° C. × ⅗.

Fig. 5. Section of sporulating lesion above, at 12 μ. × 250.

(The writer is indebted to Mr E. T. Scott for assistance with photography.)

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OBSERVATIONS ON THE SPOTTING OF TOMATO FRUITS BY *BOTRYTIS CINEREA* PERS.

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(With Plates XIII and XIV and 2 Text-figures)

THIS paper describes a characteristic spotting of both field and glass-house-grown tomato fruit which has been recorded in Great Britain for a number of years (Walton, 1937)[1]. The nature of the causal agent was the subject of much surmise until recently when Read (1937) demonstrated experimentally that this spotting was caused by *Botrytis cinerea*, and suggested the name "water spot" for the disease.

In the past *Botrytis* spotting has been confused with the stigmonose of tomato fruit caused by aphides (see below, p. 319), first recorded in this country by Bewley (1923).

SYMPTOMS

On green unripe fruit a typical *Botrytis* spot consists of a minute brownish puncture at the centre of a pale green or silver-coloured circle varying in diameter from 0·2 to more than 0·5 cm. (the spots are at first small but increase in size as the fruit swells). The area within the circle may be of normal green appearance or paler in colour than the rest of the fruit (Pl. XIII, fig. 1), and there is often a slight swelling around the central spot. There are many variants of this typical spotting. There may be two or more concentric circles (Pl. XIII, figs. 2, 3), two or more adjacent spots may coalesce (Pl. XIII, fig. 2), the spots may be entirely silvery in appearance (Pl. XIII, fig. 1 (left-hand fruit)), or the surrounding circle may be very inconspicuous or even absent as in Pl. XIII, figs. 4, 5, where circular areas, darker green in colour than the rest of the fruit, surround the central punctures. If the silvery rings are absent the spots become less conspicuous as the fruit ripens, but when rings are present they remain prominent on the ripe fruit and appear yellow in colour. The spots usually occur more frequently at the calyx end of the fruit (Pl. XIII, figs. 1, 2), i.e. in the position where floating spores are

[1] Rather similar spots ("bird's-eye" spots) of unknown origin were found associated with *Bacterium vesicatorium* spot of tomato fruit in Indiana by Gardner and Kendrick (*J. agric. Res.*, **21**, 127, 1921 and *Phytopathology*, **13**, 312, 1923).

most likely to lodge, but occasionally severe spotting of the blossom end is seen (Pl. XIII, fig. 4), due to the infection of a persistent dead corolla by *Botrytis* instead of the more usual *Penicillium* sp.

To account for the spotting the following explanation is suggested. Air-borne *Botrytis* spores settle on the surface of the fruit. Under conditions of temporary high humidity these spores germinate, the germ tubes penetrate the epidermis killing the cells around the points of entry but, with the return of less humid conditions,the sporelings are desiccated so that no fungus can subsequently be isolated. The germ tube has, however, secreted pectinase enzyme into the region penetrated and, as this enzyme diffuses outwards, the middle lamella between the epidermis and the underlying cells is destroyed and the ring surrounding the spot appears.

This hypothesis has not been disproved by the experiments, now to be described, designed to test it or to explain the success of the method finally developed for producing the spots experimentally.

METHODS

The experimental work was done during the spring and summer months with tomato plants of the variety E.S. 1 grown in 6-in. pots in a well-ventilated greenhouse. The plants were usually "stopped" after setting two trusses, but, occasionally, a third truss was allowed to set. The truss to be inoculated, trimmed to bear four to seven fruits, the largest being 2·5–3·0 cm. in diameter, was sprayed with a suspension of *Botrytis* spores ("sprayed") or the fruits were dusted with dry spores by means of a camel-hair brush and given no further treatment ("dusted"), or subsequently sprayed with water ("dusted and sprayed"). The sprayed or dusted truss was then introduced into a sterilized wide-mouthed (bolthead) flask containing a little water, the mouth plugged with cotton-wool and the flask held in position by wiring it to the stake supporting the plant (see Pl. XIV, fig. 5). The plant was then well watered. After 16–17 hr. the flask was removed and the truss kept under observation. Spots had frequently appeared by the time the flasks were removed and the fruit was usually re-examined during the next 24 hr. and again at intervals to observe the growth in size of the spots. The inoculations were usually made in the evening and the flasks removed the next morning, and it was customary to keep drops of the spore suspension employed on slides in a moist chamber overnight and to estimate the spore germination the next morning. Experiments confirmed the fact that *Botrytis* spores germinate less readily with age and,

for most experimental inoculations, the spores employed were taken from 7 to 10-day-old cultures on malt or potato agar.

During 1936 the details of this method were being worked out, and its success may be seen by comparing the 1936 and 1937 results which are set out in Table I.

Table I

Summary of experiments with Botrytis cinerea

Method of inoculation	No. of trusses treated	No. of fruit inoculated	No. of fruit spotted	% spotted
1936				
Sprayed	21	76	41	54·0
Controls	6	19	2	10·5
1937				
Sprayed	30	171	147	—
Dusted	9	42	34	—
Dusted and sprayed	6	26	25	—
	45	239	206	86·2
Controls	24	118	7	6·2

For record purposes a fruit was scored as "not spotted" or "spotted", with a note as to the severity of the spotting, and the actual number of spots was not usually counted. *Botrytis*-treated fruit generally developed a large number of spots, while a control fruit, spotted as the result of contamination by air-borne spores, developed only two or three spots (see below, p. 316).

Factors which influence spotting

(1) *Fruit size.* When the 1936 results were rearranged according to the size of fruit inoculated (see Table II) it became evident that increased resistance to *Botrytis* attack accompanied increase in size of the fruit.

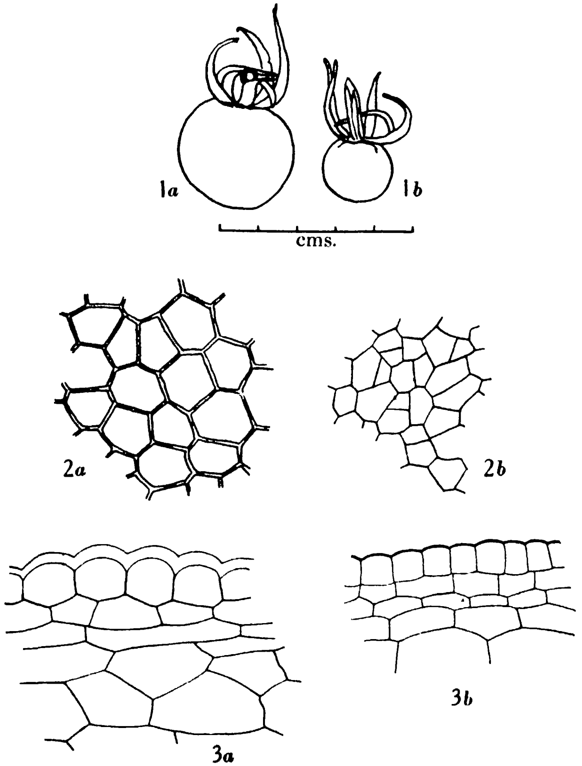
Table II

Effect of size of fruit on spotting

Size of fruit	No. inoculated	No. spotted	% spotted
Large (diam. >3 cm.)	28	1	3·6
Medium (diam. 1·5–3·0 cm.)	16	11	69·0
Small (diam. 0·75–1·5 cm.)	32	29	90·8

Anatomical investigations showed that the increased resistance could be correlated with increased thickness of the epidermal cell walls. Text-fig. 1 shows examples of susceptible and resistant fruits and indicates their anatomical differences (Text-fig. 1 should be compared with

Text-fig. 2, which shows the extremely thickened epidermal layers of a still older fruit). The actual size of a fruit is not always a reliable indication of the fruit's susceptibility to spotting, but increased resistance accompanies the rather sudden change of the fruit surface from a slightly matt to a darker green glossy appearance which occurs during the swelling of a fruit.



Text-fig. 1. Resistant and susceptible tomato fruits. 1, Whole fruit; 2, surface view of epidermis; 3, transverse section of epidermis; *a*, resistant fruit; *b*, susceptible fruit. Detail × 305.

(2) *Turgidity of fruit.* In the early stages of this investigation attempts to inoculate detached fruit kept in a moist chamber met with little success. Comparative trials then showed that fruit on the plant was more easily and more intensely spotted than similar detached fruit when sprayed with the same spore suspension (see Table III, section (*a*), and top line of section (*c*)). It was suspected that this difference in

behaviour could be accounted for by the greater turgidity of fruits still attached to the plant. To test this idea two groups of similar plants were taken and water was withheld from one group until the plants were severely wilting when, after the trusses of both series had been sprayed with the same spore suspension and bottled overnight, normal watering was resumed. It was found that fewer fruits on the wilting plants were spotted and that the spotting was less severe than on turgid plants. The experiment was repeated with similar results, and the two experiments are summarized in Table III, section (*b*).

Table III

The effect of fruit turgidity on spotting

	No. fruit inoculated	No. spotted	% spotted	
Attached	27	13	48·3	*a*
Detached	15	3	20·0	
Plant wilting	51	18	35·3	*b*
Plant turgid	33	27	82·0	
Detached (blossom-end in water)	39	4	10·2	*c*
Detached (calyx-end in water)	41	40	97·5	

Sections *a*, *b* and *c* in the above table are the totals derived from comparable pairs of experiments.

Further trials were made with detached fruit in a moist chamber comparing the effects of placing the blossom-end and the calyx-end (with or without removing the calyx) in water, when it was found that if the calyx-end dipped in water severe spotting occurred. Fruits left overnight with their calyx-ends in water became very turgid, and on account of this great turgidity water-filled blisters developed instead of typical spots. Uninoculated control fruits did not develop blisters, but medium-sized fruits often split.

A possible explanation of the decreased susceptibility of fruit not fully turgid is that, as the penetration of the fungus is purely mechanical and the epidermis slightly elastic, penetration is more easily effected when the epidermis is tightly stretched.

(3) *Variety of tomato.* From general observations some evidence accumulated to show that certain varieties are more susceptible than others to *Botrytis* spot and, though varietal resistance is a factor of minor importance and without practical significance, it was thought worth while to record the following details.

Counts were made of the numbers of plants carrying naturally spotted fruit in four consecutive rows, two of the variety Ideal and two of a Stirling Castle hybrid resistant to leaf mould (*Cladosporium fulvum*), in

one of the experimental houses on the Station nursery. It was found that thirteen of twenty Ideal plants and six of twenty Stirling Castle carried spotted fruit and, in the adjacent house where the experiment was duplicated, the corresponding figures were seventeen of twenty and five of twenty, making the totals thirty plants with some fruit spotted out of forty for Ideal and eleven out of forty for the Stirling Castle hybrid. Also, the Ideal fruits were more heavily spotted than those of Stirling Castle. This suggested a difference of susceptibility between the two varieties and additional evidence was obtained from the results of the experimental inoculations which are summarized in Table IV.

Table IV

Varietal susceptibility

Variety	No. fruit inoculated	No. spotted	No. spots per fruit
Ideal	15	10	20·4*
Stirling Castle hybrid	14	10	11·4*

* The difference between these numbers is not statistically significant.

In another experiment fruits on similar plants of the varieties Ailsa Craig, E.S. 1, Radio and Potentate were inoculated at the same time under the same conditions when, as judged by the eye, there was no difference between the degree of spotting on E.S. 1 and Ailsa Craig, but Radio was less severely and Potentate more severely affected than the first two varieties.

This difference in susceptibility is probably related to differences in the resistance of the epidermal layers but, although fruit of the Stirling Castle hybrid and Radio are seen to have thicker fruit walls than Ideal and Potentate respectively when cut open longitudinally, no definite differences in the structure of the epidermis was observed in freehand sections cut from similarly sized fruits.

(4) *Humidity.* Several experiments were performed to study the degree of humidity necessary to allow spore germination to take place and for spotting of the fruit to result. In the first experiment six plants, each bearing one truss, were taken and the fruit dusted with dry spores. One of the trusses was then enclosed in a flask containing distilled water in the usual manner while, in the flasks enclosing the other trusses, the water was replaced by sulphuric acid diluted to give relative humidities of 90, 80, 70, 60 and 50%. When the flasks were removed the fruit enclosed over water (i.e. subjected to a R.H. of 100%) was covered with a thin film of moisture and spots developed on these fruits, while the

surface of all the other fruit was dry and no spots developed. In subsequent experiments fruit was lightly sprayed with a spore suspension and then enclosed in atmospheres of varying relative humidity when, overnight, spotting occurred if the R.H. was 90 % or higher. In one series kept under constant observation the spots of water completely dried off the fruits kept at 80–60 % R.H. in about 5 hr., i.e. dried before penetration of the fruit had occurred.

(5) *Length of time necessary for infection.* To determine the length of time necessary for infection trusses on seven to ten similar plants were sprayed with a spore suspension, enclosed in flasks, and then after varying intervals of time a plant was taken at random, the flask removed and, if necessary, the evaporation of the drops of water remaining on the fruit encouraged with an electric fan. The time from the beginning of the experiment to the disappearance of the drops of water on the fruit was noted. When drops of water remained on the fruit less than 4 hr. no spotting resulted; if they persisted 4–5 hr. a few spots developed, and if 6 hr. or longer the fruit was more heavily spotted. This result was obtained with an average temperature of 18·9° C. (66° F.) during the first 4 hr. of the experiments and when the spores used germinated in water on a slide after 2–3 hr. The germination of the spores in distilled water is only a rough guide as to their behaviour on the fruit. In one of the experiments drops were removed from the fruit and examined after 4 hr. when the length of an occasional germ tube was six times that of the spore, whereas on the slide the length was less than twice that of the spore.

Occasionally, if a large drop of water persisted 18 hr. or longer a soft rot of the fruit set in and this was more marked if the spore suspension was made in a nutrient solution. In other cases, however, when fruit was incubated for periods up to 48 hr. no soft rot resulted, which rather suggests that unripe fruits show a natural resistance to the development of *Botrytis* after penetration. This point was not investigated, but it is possible that this internal resistance is also related to the turgidity of the fruit (cf. the work of Brown (1934) and his collaborators).

(6) *Length of time spores lie on the fruit.* An experiment was carried out to determine how long a spore might lie on a fruit and retain its ability to cause a spot when conditions favourable for germination occurred. One morning seven trusses were sprayed with a spore suspension, the drops of which dried off the fruit within an hour. At intervals one of the plants was taken at random and the sprayed truss enclosed in a flask overnight. The results of this experiment are set out in Table V.

Table V

Length of time spores lie on fruit before "incubation"

When "incubated"	No. fruit	No. spotted	% spotted
Same day	6	6	100·0
After 1 day	6	5	83·5
,, 2 days	10	10	100·0
,, 4 ,,	7	5	71·5
,, 6 ,,	8	4	50·0
,, 7 ,,	3	1	33·3

The plants were carefully inspected each day and no fruit developed spots before being "incubated", and the seventh unincubated truss was unspotted on the eighth day. After 4 days the percentage of spotted fruit declined, and it is interesting to note that it was the largest fruits on the trusses incubated on the fourth to seventh days that failed to spot. It is clear, therefore, that a spore may remain on a suitable fruit for at least a week and retain its ability to cause a spot, and that, although *Botrytis* spores are viable for at least 6 months (Wilson, 1937), it is the growth of the tomato fruit which determines the period of susceptibility.

(7) *Effect of fungicides on spotting.* Three plants were well sprayed with Bouisol and oil at the strength used against leaf mould, and the next day the trusses on these plants and on three similar untreated plants were sprayed with a spore suspension and incubated. Spots developed on both sprayed and unsprayed fruit, but the spots were fewer on the fruits previously treated with the colloidal copper and oil spray.

TESTS TO DETERMINE THE ABILITY OF *BOTRYTIS* SPECIES AND OTHER FUNGI TO SPOT TOMATO FRUIT

Strains of *Botrytis cinerea* Pers. isolated from tomato fruit and stems, *Anemone*, *Statice*, *Zinnia* and fig, caused equally vigorous spotting and, in most of the experimental work, the strain isolated from tomato fruit was used.

Botrytis Tulipae (Lib.) Lind., *B. Allii* Munn and *B. Fabae* Sard. all proved able to cause similar spots to those produced by *B. cinerea* in spite of the fact that, of these four species, only *B. cinerea* caused a soft rot of green tomato fruits when introduced into a wound. *B. Fabae* and *B. Tulipae* gave rise to particularly large spots (Pl. XIV, fig. 1), and this is probably related to the fact that the spores of these species are larger

than those of *B. cinerea*. The results of experiments with *B. Paeoniae* Oud. were inconclusive.

Penicillium sp., isolated from tomato fruit, failed to cause spotting.

Phytophthora infestans (Mont.) de Bary. Field-grown tomatoes attacked by blight and also showing *Botrytis* spots were examined but, under experimental conditions, conidia of *P. infestans* gave rise to a superficial or more general rot but no spots. It is interesting to note that strains of *P. infestans* obtained on potato from Kirton, Lincolnshire and Scotland vigorously attacked potato plants but failed to cause any damage to tomato fruit or foliage, while a strain attacking field-grown tomatoes in Jersey caused a vigorous rot of all the tomato plants and fruit inoculated.

Phytophthora parasitica Dast. Zoospores produced no spots but a general rot (buck-eye rot).

Cladosporium fulvum Cooke. Particular interest is attached to this fungus because of its ubiquitous occurrence on tomatoes in this country, and Read (1937) was originally of the opinion that *C. fulvum* could cause a type of spotting, but this view was not confirmed by the results of the later experiments which are summarized in Table VI.

Table VI

Experiments with Cladosporium fulvum

Fungus	No. fruits inoculated	No. spotted	No. of spots per fruit
C. fulvum	133	20	1·5
B. cinerea	56	49	Many
Controls	108	7	1·3

Unfortunately, it was during certain of the experiments with *Cladosporium* that the greenhouse became contaminated with *Botrytis* spores derived from a neglected *Pelargonium* plant. Eighteen of the spotted fruit in the *Cladosporium* series and the seven in the control series all occurred in four experiments carried out during one week, and evidence that these spots were the result of contamination was afforded by the facts that the number of spots per spotted fruit was very few (see Table VI), the spots were all on the calyx-end of the fruit, a few untreated fruits developed spots at that time, and the trouble ceased with the removal of the diseased *Pelargonium* mentioned above. Of eight further independent experiments, seven gave negative results although in some the incubation period was extended to 24 hr. (longer than the time necessary to initiate leaf infection by *Cladosporium*), and the plants

were kept under observation for a fortnight. In one experiment two fruits of nine inoculated each developed a few typical *Botrytis* spots.

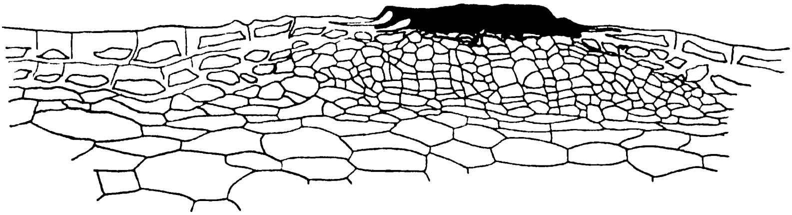
In a few supplementary experiments with detached fruit standing in water, spots or blisters developed on fruit sprayed with *B. cinerea* spores when none occurred on the fruit inoculated with *Cladosporium fulvum.*

The *Cladosporium* spores used were taken from carefully selected young natural infections and, though contamination with *Botrytis* was possible, it was thought to be very unlikely.

It was concluded from this study that *Cladosporium fulvum* does not cause a spotting of tomato fruit and, from *a priori* considerations, if it did fruit spotting should be of more frequent occurrence.

PATHOLOGICAL HISTOLOGY

Penetration of the fungus was observed by mounting epidermal strips in lactophenol and staining with cotton blue and by double staining with thionin and orange G (Stoughton's method) microtome sections cut at 10–20μ. of material fixed in weak Flemming's solution.



Text-fig. 2. Transverse section through the centre of a spot 35 days after inoculation. ×172.

The germ tube develops a penetration peg and enters a single epidermal cell which collapses, and the contents of the adjacent cells become granular. This group of cells is killed, turns brown (Pl. XIV, fig. 2) and so causes the central puncture. It was noticed that when the spores were dusted on to the fruit the germ tubes were much shorter than when the spores were sprayed. In the latter case the germ tubes usually grew to a length of five or more times that of the spore before penetrating the epidermis (Pl. XIV, fig. 2) while the germ tubes from dusted spores were short and penetrated almost immediately, possibly because of their closer contact with the epidermis. In experimental infections, when the number of spores applied was large, single lesions caused by the penetration of several germ tubes were often observed.

The penetration wound is superficial and, usually, not more than two layers of cells below the epidermis are involved. The pale ring surrounding the puncture is caused by a layer of air between the epidermis and the underlying tissue, but, in stained preparations, the epidermal cells surrounding the central puncture have appeared normal.

Sections were also cut of spots fixed at varying intervals up to 35 days after inoculation. No trace of mycelium was detected in material fixed 4 days after inoculation when a few cells below the site of penetration were seen to be dividing and, later, a well-developed layer of wound tissue is laid down (see Text-fig. 2). It is this wound tissue which causes the swelling often seen in the centre of an old spot.

Experiments with enzymes

Attempts were made to produce spots by means of pectinase enzyme prepared from the germ tubes of *Botrytis cinerea* by Brown's method (1915). After testing the activity of an enzyme preparation, by observing its ability to disintegrate thin slices of carrot and potato tuber, drops of the enzyme were placed on the surface of tomato fruits both on the plant and detached and either in a moist chamber or not. The surface of the fruit beneath the drops was then pricked with a very fine insect pin fitted with a cork shield so that the depth of the puncture was not more than about 0·2 mm., or a larger wound was made with a dissecting needle. It was found that drops of enzyme placed on the surface of the fruit without injury to the underlying epidermis caused no damage even when they persisted for more than 24 hr. If the enzyme was boiled for a few minutes before applying it to the fruit the puncture below a drop differed in no way from a control puncture through a drop of distilled water but, when the puncture was through untreated enzyme, the surrounding tissue turned brown within 12 hr. and a small brown spot similar to, but larger than, that at the centre of a natural infection resulted whether the fruit. was enclosed in a moist chamber or not (Pl. XIV, fig. 3). Occasionally, a very faint ring developed around the puncture but, as soon as the green fruit began to ripen, the spot became prominent because a circle of tissue around the puncture ripened more slowly (Pl. XIV, fig. 4).

As can be seen from the figures, the resemblance of spots produced by the enzyme to natural spots is not very close but the difference may perhaps be accounted for by the manner in which the enzyme was introduced. The ring which results from a natural infection is undoubtedly a

diffusion phenomen but no explanation of the exact mechanism of ring formation can be offered.

The only difference between enzyme spots on attached and detached fruits was that as an attached fruit increased in size the centre of the spot became slightly depressed.

Enzyme prepared from *B. Fabae* differed in no way from the *B. cinerea* enzyme and caused similar effects on the fruit. This suggests that the larger sized spots associated with *B. Fabae* are due to the larger amount of enzyme introduced.

EXPERIMENTS WITH SUNLIGHT

One explanation of the origin of *Botrytis* spots was that sunlight focused on to the surface of the fruit by means of water droplets damaged the epidermis but experiments to test this explanation have given negative results. At different times a total of thirty-three fruits on seven trusses were exposed near the glass to direct bright sunlight during June and July. The plants were sprinkled with tap water and, when this dried, sprinkled again, but no damage resulted.

Walton (1937) was of the opinion that sunlight caused the spotting, but his experimental evidence is open to an alternative explanation, since the repeated damping over of the plants would make conditions very suitable for *Botrytis* attack to occur. In this connexion it may be noted that natural spotting frequently occurs on lower trusses never exposed to direct sunlight, but subject to very humid conditions, and that although strong sunlight is able to scorch the leaves of soft plants, and is a contributary cause to "green-back" of fruit, no evidence has been obtained that it damages the fruit in any other way.

STIGMONOSE

For several years we considered insects to be the cause of *Botrytis* spot, but Read (1937) has shown experimentally that the aphid *Myzus convolvuli* Kalt. (=*M. pseudosolani* Theob.) is able to damage tomato fruit and that this damage differs from *Botrytis* injury (see Pl. XIII, fig. 6 and compare with figs. 1–5). It was proposed that the name stigmonose, first applied by Bewley (1923) to aphid damage of tomato, should be retained for aphid injury which occurs naturally but infrequently. The spots caused by aphides are pale in colour, slightly raised with margins less clearly defined than those of spots caused by *Botrytis*, and with, or without, a very minute dark central puncture. Examination of the fruit usually shows traces of aphides (Pl. XIII, fig. 6). Cast skins

may remain attached to the fruit, particularly around the calyx which often shows a yellow mottle.

Control

It will be clear from the above results that, for *Botrytis* spotting to occur, *Botrytis* spores and excessive humidity are necessary. The source of *Botrytis* spores is usually infected snags and plant debris resulting from careless pruning and, under glass, the excessive humidity, which also encourages *Botrytis* rot, may be caused by careless watering and ventilation. *Botrytis* spotting usually reflects faulty cultural methods and its control is, therefore, largely a matter of employing the best horticultural practice. Stigmonose can, if necessary, be controlled by fumigation.

Summary

Characteristic ring-like spots caused by *Botrytis cinerea* on tomato fruits are described, with observations on certain factors which influence the spotting.

Evidence is presented to show that, under conditions of high humidity, *Botrytis* spores lying on the surface of immature fruits germinate, penetrating the epidermis, and that the spots result from the pectinase enzyme secreted by the germ tubes. With the return of drier conditions the sporelings die and no fungus can be isolated from mature spots.

It was found that similar spots could be produced experimentally by other species of *Botrytis* but not with various other fungi able to attack tomatoes.

The *Botrytis* spots are compared with aphid injury and notes are given on control.

We wish to thank Mr G. Cockerham, Mr E. R. Wallace, and Dr T. Small for material of blight, and Mr W. C. Moore for records of the disease.

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Fig. 1.

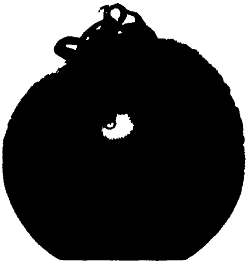

Fig. 2.

Fig. 3.

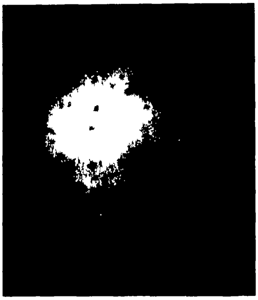

Fig. 4.

Fig. 6.

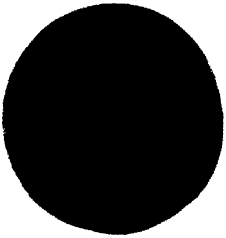

Fig. 5.

AINSWORTH, OYLER AND READ.—OBSERVATIONS ON THE SPOTTING OF TOMATO FRUITS BY *BOTRYTIS CINEREA* PERS. (pp. 308–321)

Fig 1

Fig 2

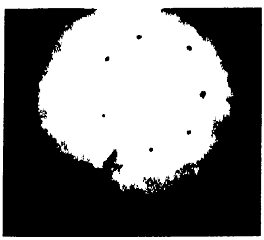

Fig 3

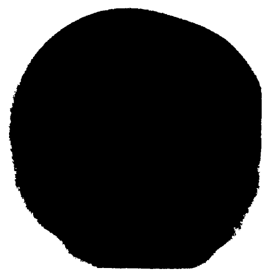

Fig 4

Fig 5.

AINSWORTH, OYLER AND READ Observations on the Spotting of Tomato Fruits by *Botrytis cinerea* Pers. (pp. 308–321)

EXPLANATION OF PLATES XIII AND XIV

PLATE XIII

Fig. 1. Natural infection, unripe fruit.
Fig. 2. Natural infection, ripe fruit.
Fig. 3. Experimental infection with *B. cinerea*, unripe fruit.
Fig. 4. Natural infection of blossom-end, unripe fruit.
Fig. 5. Experimental infection with *B. cinerea.*
Fig. 6. Stigmonose caused experimentally by *Myzus convolvuli* Kalt.

PLATE XIV

Fig. 1. Experimental infection with *B. Tulipae.*
Fig. 2. Germ tube penetrating epidermis, 16 hr. after inoculation. (Temporary mount in lactophenol stained with cotton blue.) ×460.
Fig. 3. Spots resulting from punctures with an insect pin through drops of enzyme. Control punctures alternate with the spots.
Fig. 4. Enzyme spots and control punctures on half-ripe fruit. (The punctures were made with a dissecting needle.)
Fig. 5. Method used for "incubating" experimental infections.

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EXPERIMENTAL SPAWN AND MUSHROOM CULTURE

II. ARTIFICIAL COMPOSTS

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(With Plates XV and XVI)

CONTENTS

Introduction

The experimental results of preliminary tests for the growth of different species of *Psalliota* on artificial composts have been described (Cayley, 1937), but the details of the proportions of the ingredients and the methods of composting were deferred to a later publication. In the previous paper, the records of tests with three wild species and several varieties of cultivated mushrooms on well-composted stable manure showed definitely that the wild species *Ps. campestris*, the wild grassland species generally known as *Ps. arvensis*, and the wild haystack mushroom (sp. undetermined) would not grow on stable manure compost. Experiments with artificial composts were then started with the object of finding some suitable medium for the growth of the wild species.

The main object of this paper is to supply details as to ingredients and methods of treating artificial composts, to describe further more extensive experiments, and to discuss the suitability of the various composts for the growth of the cultivated varieties of mushroom.

It must be pointed out however, that over and above laboratory

tests in large tubes and battery jars, the composts to be described below have, for the most part, only been tested in large 12 in. pots and a few larger boxes, owing to lack of space and adequate accommodation. The results, however, show that previous high temperature fermentation of the compost is not essential for the growth of the mushroom itself, although in the case of the saprophytic cultivated varieties, previous fermentation may make the necessary nutritive ingredients more easily available to the fungus. On the other hand, when dealing with manure or any other material containing a high percentage of plant residues, composting is essential in order to reduce the compost to a suitable physical condition for mushroom growth and to prevent excessive heating and burning. Where artificial heat is not available, the heat generated during composting and retained in the bed is of importance.

American workers (Waksman & Nissen, 1931; Waksman & McGrath, 1931) and others (Falck, 1928) investigated the chemical changes which take place in manure during fermentation and after the growth of the mushroom, and found that during the process of high-temperature composting of stable manure there is a considerable increase in the relative lignin content of the heap, and that after the growth of the mushroom this lignin content was greatly decreased. The American investigators concluded that the mushroom, in some way, utilized a considerable amount of lignin, more especially as a similar depletion of lignin occurred in sterilized stable manure and, hence, could not be attributed to the agency of other micro-organisms. They also held that "the need for the composting of manure, in order to develop a favourable medium for the growth of mushrooms, consists in bringing about an enrichment in the lignin and protein content and possibly a change in their chemical nature" (Waksman & Nissen, 1932, p. 272). They considered, also, that lignin and protein are the most essential nutrients for the mushroom. Falck (1928) also observed the same increase in lignin content brought about by high temperature fermentation. These points will be discussed more fully later.

INGREDIENTS FOR ARTIFICIAL COMPOSTS

A number of different artificial composts for manure and mushroom growing have been devised by various investigators with variable results which cannot be considered in detail here (Demelon & Burgevin, 1932, 1935; Demelon *et al.* 1935; Hébert, 1911; Hein, 1930 *a*, *b*; Hutchinson & Richards, 1921–3; Lambert, 1929; Paterson-Hart, 1936; Waksman & Reneger, 1934).

In view of the fact that, up to the present time, stable manure has been almost exclusively used for mushroom beds by the trade and private growers, the ingredients for the artificial composts described in this paper were based on the food of horses, namely, hay, grass and crushed oats, with the addition of straw. These ingredients are easily obtainable in this country and at a low cost.

Proteins and a complete plant food are supplied by the hay and grass, and a liberal addition to the lignin supply is introduced by the straw. The crushed oats were only used in the standard medium for spawn growing (Cayley, 1937) and not in the composts prepared in bulk.

FERMENTED HIGH-TEMPERATURE COMPOSTS

The first series of experiments were made with fermented and composted straw and hay treated as for stable manure. In bulk the composts generated a considerable amount of heat only little short of that of fermenting stable manure, but it was found that the artificial composts did not retain the heat for so long a period. This may be due to physical conditions, but the capacity for heat retention has not been investigated.

The treatment of one compost during fermentation is set out in some detail below as a standard method of composting. All the other fermented composts were treated in much the same way, but the temperature of some rose higher than others and the composts had to be turned at shorter intervals.

Artificial Compost I

Ingredients:

Two trusses of wheat straw.
Two trusses of hay (2 years old).
One large sack of chopped straw.
One large sack of chopped hay.
1 lb. sulphate of ammonia.
4 lb. slaked lime.
½ lb. phosphate of potash.

Stack in thin layers in the following order: long straw, chopped hay, long hay, chopped straw, damping each layer thoroughly with a fine rose can.

Stack to about 3 ft. and keep the surface of the stack moist by sprinkling with a fine rose can.

Leave stacked till the temperature rises to 110–120° F.

First turning. Turn over and shake to loosen any dry lumps of hay, taking care to place the outer layers in the centre of the stack. Water during this process with a solution of 2 oz. sulphate of ammonia to the gallon of water until 1 lb. has been added

in all. More water may be added if necessary, as the compost at this turning must be thoroughly moistened but not made too wet.

Stack to 3 ft. and leave till the temperature has reached its maximum (130–140° F.) and has begun to fall.

Second turning. Turn again in the same way. The compost at this stage may smell rather rank and present a very mouldy appearance. Stack and leave till the temperature begins to fall.

Third turning. Turn again and add 4 lb. slaked lime and ½ lb. phosphate of potash in powder form, distributing it as evenly as possible throughout the compost, and stack again. The rank smell will disappear after the addition of lime and the temperature will rise rapidly to about 150–155° F.

Fourth turning. Turn when the temperature has begun to fall and make up the permanent bed at this turning. Heap the compost to about 6 in. above the required level to allow for settling. Press down the compost gently but not too firmly. This is best done with a potato fork. After settling, the bed should not be more than 8 in. in depth. Press again about once every 2 days till the compost is sufficiently firm. Keep a thermometer plunged in the centre of the bed. The risk at this stage is that the bed may dry out or heat in the middle. If the temperature drops unduly fast, this indicates that the bed is too dry in the centre and must be lightly forked up, a little water added and remade. The temperature at this stage should drop gradually. Spawn at 70–75° F.

If dry spawn is used it should be moistened before planting; this reduces the incubation period between spawning and cropping by about 3 weeks.

After 3–4 weeks (or longer) the bed can be cased with soil containing 5% lime to a depth of 1½ in. Should the compost be rather on the wet side casing should be deferred till the surface has dried somewhat.

The moisture content of the compost when the bed is made up, is of the utmost importance; it should be felt in the hand at each turning and water added with discretion. A fine rose can should be used throughout.

This compost is more suitable for small rather than large beds. Ridge beds were not tried. In bulk it was not satisfactory when tested under cover in a cold cellar. It was not sufficiently broken down and friable and settled too firmly. A bed 14 sq. ft. and 10 in. deep was tested with bought commercial spawn; but unfortunately the spawn was of poor quality; the crop on the control bed was much below the average and only a few medium-sized pilei developed on the compost bed.

The temperature registered in the compost bed 1 month after spawning was 60° F.

In smaller bulk, however, in a wooden box $2 \times 1\frac{1}{2}$ ft., spawned with the same but a different sample of commercial spawn at 70° F. and kept under warmer conditions in a greenhouse, produced a good flush after 10 weeks and a second (Pl. XV, fig. 9) some 14 days later. The box was then discarded. This box was watered with ½ oz. phosphate of potash to the gallon of water after spawning.

This result suggested that the physical conditions, such as moisture content, and aeration and that the general conditions for growth in the larger bed were not satisfactory.

The two wild grassland species and the wild haystack mushroom were also tested in smaller beds in the cellar but failed to make any growth.

In the open this compost gave better results. Working in collaboration with the author, a mushroom bed of the same ingredients and treated in the same way, was made up in the open in November by Miss F. M. Durham at Otterton, Devonshire. The compost was turned four times and reached a temperature of 150° F. The bed was spawned at 70° F. with oatmeal-agar cultures of two cultivated varieties, and protected against winter weather conditions with straw, sacking and corrugated iron. Some 5–6 months after spawning, this bed produced a fair crop of several flushes.

A second compost was treated in bulk.

Artificial Compost II

Ingredients:

Two sacks chopped straw.

Two sacks chopped hay.

6 oz. sulphate of ammonia.

2 lb. slaked lime.

½ lb. phosphate of potash.

Turned four times during the course of 9 days on account of the rapid rise of temperature and reached a maximum of 165° F. on the eighth day. On the ninth day it was divided and made up into four small beds in a wooden frame with glass partitions. On the tenth day the temperature dropped rapidly and it was found that the compost was too dry. It was forked up, 1 oz. of lime and a 2 gall. pail full of freshly cut lawn mowings added to each bed and watered. This treatment raised the temperature, which varied in the four beds from 78 to 95° F. and the pH from 7·95 to 8·1.

Two beds were spawned with oatmeal-agar cultures of two cultivated varieties (at 72 and 82° F.), a third with an oatmeal-agar culture of the wild haystack mushroom (at 81° F.), and the fourth left unspawned as control.

This compost also gave very unsatisfactory results, but nevertheless one of the cultivated varieties produced a good clump of normal pilei 4 months after spawning.

These preliminary tests in bulk confirmed the tests made in large tubes and battery jars under more controlled conditions and showed that the cultivated varieties could develop normal pilei on a fermented medium other than stable manure, consisting of straw and hay.

The wild haystack mushroom which has been shown to be intermediate in its physiological properties between the two wild grassland species and the cultivated varieties (Cayley, 1937) failed to grow in beds of Composts I and II. In battery jar tests however, under more strictly controlled conditions, it was found to tolerate Compost II if sterilized on the fourteenth day and further fermentation arrested. The wild grassland species were not tested on this compost.

Compost I was repeated the following year and again tested in the cold cellar. This time the phosphate of potash was omitted and only 12 oz. of sulphate of ammonia added. Half the compost was treated with lime and the other half left without; it was turned four times and placed in the permanent bed at the fourth turning. The maximum temperature attained with lime was 130° F. (pH 8·08) and that without lime 124° F. (pH 8·49).

In general, the addition of lime to a compost fermented at a high temperature produces little or no effect on the pH. As can be seen in Table I, in composts without soil it has not increased the alkalinity above that of the same compost without lime, except in Division II by 0·02, and Division III by 0·03. In unfermented composts, on the other hand, the addition of lime does affect the pH, but not to any marked degree.

Beds of this compost were tried in the cold cellar, and spawned in November with proper spawn of a cultivated variety 9½ months old. The low temperature of the cellar was not conducive to rapid growth. The following May numerous incipient fruiting bodies appeared on both beds but few reached maturity. After 9 months the beds were forked up and it was found that the compost had settled too firmly and that aeration was totally inadequate. In both beds, with and without lime, the compost was sticky and the spawn had not spread evenly through the beds but occurred in patches and was stringy. Apart from being sticky and too firm, the compost had no rank smell and the water content appeared to be satisfactory. In large pots or boxes on the same composts, cultures of the cultivated varieties, spawned with spawn 8 months 10 days old, produced normal pilei (Table I, Composts III, IV, Pl. XV, figs. 2, 10).

These results again suggested that owing to the sticky condition of these composts in bulk, the main cause of failure was lack of adequate aeration.

Low-temperature composts

The low-temperature composts mentioned in the previous paper (Cayley, 1937) were made with the same ingredients as Compost II.

Ingredients:

1 lb. chopped straw.

1 lb. chopped hay.

Mixed and moistened with 2 oz. sulphate of ammonia to the gallon of water, packed in a large biscuit tin and allowed to ferment. After 10 days ½ oz. slaked lime mixed in and the compost replaced in the tin. Maximum temperature attained 90° F.

Samples were taken out and sterilized at intervals and different periods of fermentation tested in battery jars and large tubes.

The wild grassland species failed to grow in any low-temperature compost; the wild haystack mushroom grew and fructified on a compost subjected to fermentation for 1 month but not longer, and the cultivated varieties grew well on composts subjected to fermentation of 1–2 months' duration.

Composts naturally rotted without heat

A further more comprehensive series of experiments were then made both with fermented straw, hay and dried lawn mowings, and the same ingredients allowed to rot naturally without heat in the open. For the different mixtures of these ingredients see Tables I and II.

Although it is possible that some mild form of fermentation takes place without the generation of heat in damp plant residues in the open, in this case, it was of short duration (3–4 weeks), and only a slight degree of disintegration took place before spawning. It is essential, if dried lawn mowings are used, that they should be freshly cut and thoroughly dried. They should be spread out in thin layers protected from rain and not allowed to generate any heat. If used undried they make the compost too moist and soapy.

Method of preparing naturally rotted composts

Mix the ingredients thoroughly, moisten with a fine rose can and spread out in a thin layer on a concrete or paved surface. The surface of the compost is then watered with a solution of 2 oz. sulphate of ammonia to the gallon of water, but not so heavily that the solution drains through rapidly. If the quantity of compost is small a more concentrated solution can be used. The mixture is left spread out for 10–11 days and the surface kept moist during hot sunny weather. After this interval the mixture is divided in half, slaked lime sprinkled over one half and the other half left without. Both halves should be lightly watered and left spread out for another 10 days or longer. The compost can then be made up into beds and spawned without risk of heating provided it is not used in too great bulk. An equal volume of soil is added to those composts with soil when the beds are being made up.

COMPOST TESTS WITH CULTIVATED VARIETIES

The majority of the later tests, both with fermented and naturally rotted composts, were made in large 12 in. pots and a few boxes $2 \times 1\frac{1}{2}$ ft. The pots were spawned as soon as the composts had been prepared, a period extending over some weeks from September onwards. The pots and boxes were kept in a wooden shed during the winter, covered with blanketing and a paraffin stove used to keep out the frost. The temperature of the shed during the winter months ranged from 33 to 56° F., conditions far from ideal for mushroom growing.

As controls, some of the unfermented composts were sterilized in glass battery jars and the cultures kept under aseptic conditions in the laboratory. These controls gave interesting results, as the development and spread of the mycelium in the different composts could be kept under observation. These will be dealt with later.

The results of the tests with cultivated varieties in pots on twenty-four different composts are set out in Tables I and II. It can be seen that nineteen out of the twenty-four tests were positive; of the remaining five the negative results were with Composts XI–XIII, Table I, Division III, might possibly be due partly to the toxic effect of oxalic acid and partly to unsuitable physical conditions, and the two tests, Composts VII and VIII, were made with single-spore cultures. Hence the cultivated mushroom will crop on a fairly wide range of media both fermented and naturally rotted without heat, with and without lime and soil, but the most successful results were obtained with fermented and naturally rotted composts containing both soil and lime. All the various composts proved to be on the alkaline side.

In Table I, Divisions I and II, and in Table II, Division IV, 5 g. commercial (neutral) superphosphate of lime was added per pot and watered in after spawning. In Table II, Divisions V and VI, no superphosphate was added, as all the composts showed a pH of less than 8. Fermented mixtures containing dried lawn mowings, Table I, Division III, are not to be recommended, as the resulting product is a lumpy, sticky, highly alkaline mass.

As these composts (Division III) were so alkaline and the physical condition so unsuitable, 0·5 l. of 0·01 % oxalic acid was added per pot, partly to reduce the alkalinity and possibly alter the physical condition, and partly with the object of finding out whether oxalic acid is toxic to the mushroom. The mycelium of all the forms of mushrooms under investigation is thickly encrusted with crystals of calcium oxalate when

Table I

Fermented artificial composts. High-temperature composting. Pot cultures spawned with cultivated varieties

Division I. Composts III–VI.

One large sack chopped straw, One large sack chopped hay } + 6 oz. sulphate of ammonia.
Half without lime, half with 4 oz. slaked lime.
Equal volume of soil added to those composts with soil.
17 days composting. Max. temperature 150° F.

Compost ...	III +l. −s.	IV −l. −s.	V +l. +s.	VI −l. +s.	
*p*H	8·32	8·39	7·58	7·51	5 g. superphosphate of lime added per pot, and watered in after spawning
Origin of culture	Stipe	Stipe	Stipe	Stipe	
Fructification	+ + Pl. XV, fig. 10	+ + Pl. XV, fig. 2	+ + Pl. XV, figs. 3–5	+ + Pl. XV, figs. 6, 7	

Division II. Composts VII–X.

One large sack chopped straw, One large sack dried lawn mowings } + 6 oz. sulphate of ammonia.
Half without lime, half with 4 oz. slaked lime.
Equal volume of soil added to those composts with soil.
16 days composting. Max. temperature 148° F.

Compost ...	VII +l. −s.	VIII −l. −s.	IX +l. +s.	X −l. +s.	
*p*H	8·02	8	7·57	7·57	5 g. superphosphate of lime added per pot, and watered in after spawning
Origin of culture	Single spore	Single spore	Stipe	Stipe	
Fructification	−	−	+ + Pl. XV, fig. 8	+	

Division III. Composts XI–XIV.

One large sack chopped hay, One large sack dried lawn mowings } + 10 oz. sulphate of ammonia.
Half without lime, half with 4 oz. slaked lime.
Equal volume of soil added to those composts with soil.
20 days composting. Max. temperature 160° F.

Compost ...	XI +l. −s.	XII −l. −s.	XIII +l. +s.	XIV −l. +s.	
*p*H	8·6	8·57	8·72	8·92	Watered with ¼ l. 0·01% oxalic acid after spawning
Origin of culture	Not tested	Stipe	Stipe	Stipe	
Fructification	−	−	−	+	

+l. with lime; −l. without lime. +s. with soil; −s. without soil.
+ fructification; − no fructification.

exposed to the air (Hein, 1930*c*), and hence it was thought that the mushroom might possibly be able to use free oxalic acid. Onslow states (1931, p. 113) that Dorée & Barton Wright (1927) have suggested a constitutional formula for alkali lignins, and that they maintain, that in

Table II

Artificial composts. Naturally rotted in the open without heat for 3–4 weeks. Pot cultures spawned with cultivated varieties

Division IV. Composts XV–XVIII.

One large sack chopped straw, One large sack chopped hay + 6 oz. sulphate of ammonia.
Half without lime, half with 4 oz. slaked lime.
Equal volume of soil added to those composts with soil.
Not turned.

Compost ...	XV +l. −s.	XVI −l. −s.	XVII +l. +s.	XVIII −l. +s.	
*p*H	8·12 8·07	7·93 7·9	7·25 7·3	7·28 7·28	5 g. superphosphate of lime added per pot, and watered in after spawning
Origin of culture	Same stipe culture throughout				
Fructification	+	+	+ +	+	

Division V. Composts XIX–XXII.

One large sack chopped straw, One large sack dried lawn mowings + 6 oz. sulphate of ammonia.
Half without lime, half with 4 oz. slaked lime.
Equal volume of soil added to those composts with soil.
Not turned.

Compost ...	XIX +l. −s.	XX −l. −s.	XXI +l. +s.	XXII −l. +s.	
*p*H	7·56 7·53	7·43 7·36	7·5 7·46	7·1 7·08	No superphosphate added
Origin of culture	Same stipe culture throughout				
Fructification	+	+	+	+	

Division VI. Composts XXIII–XXVI.

One large sack chopped hay, One large sack dried lawn mowings + 6 oz. sulphate of ammonia.
Half without lime, half with 4 oz. slaked lime.
Equal volume of soil added to those composts with soil.
Not turned.

Compost ...	XXIII +l. −s.	XXIV −l. −s.	XXV +l. +s.	XXVI −l. +s.	
*p*H	7·86 7·77	7·73 7·68	7·32 7·32	7·17 7·17	No superphosphate added
Origin of culture	Same stipe culture throughout				
Fructification	+	+	+	+	

Symbols the same as in Table I.

such a structure, decomposition by oxidation would probably give rise to general disruption and the production of oxalic acid and carbon dioxide.

In spite of the extreme stickiness of the compost, the "Honeymoon" variety produced small pilei after prolonged growth in Compost XIV (Table II) to which oxalic acid had been added, but doubtless the free oxalic could not have remained as such for any length of time.

No growth occurred in Composts XI, XII, and XIII.

On the other hand, the same mixture (chopped hay and dried lawn mowings) naturally rotted without heat, showed lower alkalinity, was not treated with oxalic acid or superphosphate and the cultures produced normal pilei (Pl. XVI, fig. 27).

The addition of garden soil was found to reduce the alkalinity of both fermented and naturally rotted composts, with the exception of the fermented composts in Division III (Table I). Here the *p*H is raised by the addition of soil.

p*H* OF COMPOSTS BEFORE AND AFTER STERILIZATION

These Division III composts were tested before and after sterilization, and it was found that the *p*H was raised by sterilization in composts without soil (Table I, Composts XI and XII), and more markedly in Compost XIV with soil but without lime.

In Division II (Table I) Composts IX and X were also tested before and after sterilization, and here again the *p*H was slightly reduced in both cases (Table III).

Table III

p*H of composts before and after sterilization*

Composts	Unsterilized	Sterilized
IX. Chopped straw, dried lawn mowings +l. +s.	7·57	7·4
X. Chopped straw, dried lawn mowings −l. +s.	7·57	7·53
XI. Chopped hay, dried lawn mowings +l. −s.	8·6	8·86
XII. Chopped hay, dried lawn mowings −l. −s.	8·57	8·74
XIII. Chopped hay, dried lawn mowings +l. +s.	8·72	8·7
XIV. Chopped hay, dried lawn mowings −l. +s.	8·71	8·92

AGE OF SPAWN

It will be seen in the explanation of the plates, p. 339, that spawn of varying ages was used in these compost tests, ranging from 2 months to 9 months and 3 weeks. In the pot and box cultures all the spawn was grown in the laboratory under sterile conditions on the standard spawn compost with the exception of the box in Pl. XV, fig. 9. In the pots of fermented compost in Pl. XV, figs. 1, 2, the spawn was 8 months 10 days old, fig. 3, 2 months 1 day old, and fig. 10, 7 months 11 days old, showing that, when grown in this medium, spawn remains viable for a considerable period and that the age (within these limits) has not affected the resulting crop.

MYCELIAL DEVELOPMENT IN DIFFERENT COMPOSTS

Different physical conditions in these artificial composts greatly affect the type of mycelial growth. The standard spawn compost, consisting of chopped straw, chopped hay, crushed oats and Styer's nutrient solution "A" (1928), sterilized fresh and not submitted to any decomposition by micro-organisms, is a loose open compost in which the mycelium is fine and dense, spreads evenly and rapidly, and thoroughly permeates the compost. If not allowed to dry out, the spawn will remain in the diffuse filamentous condition for a considerable period without strand formation. Again, in high- and low-temperature composts without soil, strand formation is delayed. Pl. XVI, fig. 14 is a culture of commercial spawn in a 2 months low-temperature compost after 10 weeks growth, and fig. 16 a culture of the "Honeymoon" cultivated variety in the same compost after 18 weeks growth. In the latter culture, with the exception of the course fructifying strand, the mycelium is still diffuse.

The addition of soil to high-temperature and naturally rotted composts sets up entirely different physical conditions. The composts are denser and of a more homogeneous nature, with the result that the mycelium tends to develop strands in a comparatively short time, and, hence, these composts are useless for spawn growing. Pl. XVI, figs. 11 and 12, are cultures of the cultivated mushroom in a high-temperature compost with and without lime but both containing soil in which strand formation has occurred after 11 weeks.

The effect produced by the addition of soil to naturally rotted composts is still more marked in the case of the wild *Ps. campestris*, a soil-inhabiting species. Pl. XVI, figs. 17–20, is a series of cultures of *Ps. campestris* after 3 months growth, figs. 17 and 18 without soil, figs. 19 and 20 with soil. Strand formation has occurred in the cultures with soil and not in those without.

The addition of lime together with soil tends to produce strand formation both with the cultivated varieties and with *Ps. campestris* (cf. Pl. XVI, figs. 12, 13, and 19, 20).

The absence of lime in both fermented and naturally rotted composts induces a tendency to clumping of the sporophores of the cultivated mushroom (Pl. XV, figs. 2, 7: Pl. XVI, fig. 25).

Owing to lack of adequate cultural conditions no reliable data are available as to the length of the period of incubation with the various composts, but in general, pilei of the cultivated varieties developed more

rapidly and were of better consistency on fermented than on naturally rotted composts. As regards texture, the pilei produced on artificial composts are, as a rule, neither so firm nor so heavy as on manure, although occasionally firm pilei have been obtained on fermented composts in medium bulk in the boxes.

The commercial varieties vary in flavour when grown on manure. The same is the case on artificial composts. The "Honeymoon", the best flavoured variety used in these experiments, retains its delicate flavour when grown on artificial composts.

Discussion

The investigation of artificial composts is still in the experimental stage and no claim is made that the results are, as yet, of any commercial value, except in so far as spawn growing is concerned. The standard spawn compost is cheap, easy to make and has the advantage of delaying the transition from diffuse filamentous mycelial growth to strand formation and thus enables the spawn to be kept in a suitable condition for a prolonged period. Spawn in which stringy growth predominates is known to give poor results.

The problem of the nutritional requirements of the mushroom and its reactions to the physical conditions in the substratum is so complex that it is impossible to attribute any phenomenon to any one cause or factor; but these results show definitely that the various composts, both fermented and naturally rotted, with and without lime, contain the necessary nutrients for the growth of the cultivated mushroom, and that, in spite of very inadequate cultural conditions, normal pilei developed in the majority of the mixtures tested.

The main difficulty throughout has been to secure favourable physical conditions, i.e. adequate aeration and friability, and possibly, as described by Pizer (1937*b*) for stable manure, some physicochemical condition. Samples of artificial composts of sticky consistency, which had given poor results, were submitted to Pizer, who pronounced the opinion that stickiness in artificial composts was quite a different property to "greasiness" in stable manure, and that the samples submitted to him had not been sufficiently composted. The latter is probably true, but these composts were not made primarily with the object of devising a suitable compost for growing the commercial cultivated varieties, but in order to confirm previous tests as to the reactions of the two wild grassland species *Ps. campestris* and *Ps. arvensis* and the wild haystack mushroom to fermentation of short duration; the cultivated varieties were only

used as controls to test the physical condition and the nutritional value of the various mixtures.

The stickiness in fermented artificial composts is most marked in the richer mixtures containing dried lawn mowings, both with and without soil, but is considerably less in mixtures of chopped straw and hay. The addition of an equal volume of soil to the latter mixture resulted in a good friable compost, but this was only tested in pots and boxes and not in greater bulk.

Unfortunately, Pizer's method for improving greasy stable manure by adding gypsum at certain stages during the process of composting had not been published when these composts were prepared, and it remains to be seen whether this method would alter the physical condition of sticky artificial composts. If adequate physical and physico-chemical conditions can be induced it should be possible, after further experiment, to produce a compost containing the same ingredients capable of carrying a crop equal to that on stable manure. These artificial composts do not retain the heat so long as stable manure and therefore artificial heating of the mushroom houses would be necessary if quick results are to be obtained. For instance, Pl. XV, fig. 9, shows the second flush on a compost fermented at a high temperature for 17 days and kept during the incubation period between spawning and cropping in an intermediate greenhouse. Under these warmer conditions the compost retained the heat for a much longer period. The first flush appeared 10 weeks after spawning and the second about 2 weeks later. On the other hand, in the culture in Pl. XV, fig. 10, of home-grown spawn of a white market variety kept in a cold cellar throughout, the first flush only appeared after 5 months 11 days.

The effect on mycelial growth produced by the addition of an equal volume of soil to the composts requires further investigation. The first effect is to reduce the temperature, the second to induce early formation of strands, and it is quite possible that by reducing the amount of soil a mixture could be produced in which strand formation would be sufficiently delayed to allow of the compost being thoroughly permeated with vigorous filamentous mycelium before strand formation and fructification.

Hein (1930*c*), Pizer (1937*b*) and Styer (1930) have observed that strand formation predominates in composts with too high moisture content. The early strand formation in the battery jars figured in Pl. XVI cannot be attributed to excessive moisture, as the cultures were kept on the dry side during the early stages of growth, but rather to the

reduced aeration in the denser composts containing soil; or possibly to some unknown ingredient in the soil which stimulates a more rapid rate of metabolism at the expense of diffuse vegetative mycelial growth.

When plated on nutrient agar the submerged mycelium produces few or no crystals, but the aerial mycelium is thickly encrusted with crystals of varying length and arrangement, mainly calcium oxalate (Hein, 1930*b*), and the drier the conditions the denser the mycelium and the whiter the appearance. Hein has suggested that the crystals may be a waste product of mycelial metabolism.

Crystals of calcium oxalate are only slightly soluble in water, and a possible explanation of the deleterious effects of excessive moisture may be, that under inadequate aeration together with too much moisture, the mycelium cannot get rid of waste products by crystallization. Hence the spread and rate of growth is restricted and the mycelium passes over into the next developmental phase, i.e. strand formation, before having derived the full benefit of the nutrients in the compost or accumulated and stored sufficient food material for normal fructification, with the result that the pilei are undersized or do not reach maturity.

The aeration of a compost in a glass container must necessarily be poor and not conducive to the development of normal life cycle, more especially after prolonged growth. Hence, although in a number of cultures the compost became thoroughly permeated with mycelium, only occasional pilei of the cultivated varieties have been obtained in battery jars.

The cultivated varieties differ somewhat from the wild grassland species with respect to moisture conditions during mycelial development. In well-composted, well-aerated stable manure, moist heat is conducive to rapid mycelial growth, and in the battery jars, under cooler conditions, it was found that the cultivated varieties would tolerate—within limits—a higher moisture content than the two wild grassland species.

As regards temperature, on the other hand, it is a well-known fact that in districts where the wild field mushroom is prevalent, hot dry weather in July and August followed by rain in September results in a heavy crop, whereas after a cool wet summer few or no mushrooms can be found. The wild field mushroom is mostly found in well established turf on heavy soils retentive of moisture and considerable drying out may be necessary for adequate aeration.

These observations suggest that of the two main interacting factors, moisture content and aeration, both of which affect the physical condition of the substratum, the presence of too much moisture is instru-

mental in reducing or inhibiting crystallization, and aerobic conditions are necessary for metabolism and growth.

The marked reduction of the lignin content in composted stable manure observed by Waksman and his co-workers, and the question as to how it is utilized by the mushroom are still unexplained. With naturally rotted straw and hay composts which have not undergone high temperature fermentation, the above described experiments show that previous high-temperature fermentation is not absolutely essential for normal growth, although the gradual disintegration by micro-organisms and by the mushroom itself during the incubation period may help to increase the relative lignin content to some extent. The two media—stable manure, some ingredients of which have been subjected to animal digestive juices, and naturally rotted straw and hay—must necessarily be entirely different both physically and chemically and perhaps are hardly comparable, but the examination of the plant residues in the artificial composts during mycelial growth has shown that the fungus penetrates the tissues, the mycelium is mainly *intra*-cellular and that the parenchymatous cells are the first to be attacked. The lignified tissues are only penetrated after prolonged growth. Lignin is present in the xylem parenchyma, but not in sufficient quantity for a lignin destroying fungus requiring a considerable amount of lignin as such. The expectation would be, *inter*-cellular mycelium, a heavily infected vascular system and rapid disintegration of the plant residues in the compost. This does not occur in sterilized artificial composts.

Artificial infections of sterile grass seedlings have shown that all the forms of mushroom under investigation can penetrate the living roots. The parenchymatous cells of the cortex are attacked and become completely filled with coiled mycelium, but the vessels remain more or less free.

Again, experiments described in a previous paper (Cayley, 1937) show definitely that the two wild grassland species will not grow on either fermented manure or any fermented artificial compost so far tested, but spawn grows quite freely on unfermented freshly sterilized straw and hay.

These results indicate that mainly proteins, hemicelluloses and cellulose are required by the fungus during the earlier stages of growth, but that lignin or lignin derivatives may possibly be necessary for fructification, although Waksman & McGrath (1931) found very little lignin in the pileus itself (0·92 % dry material) as compared with hemicellulose (13·66 %) and cellulose (4·86 %).

SUMMARY

Methods for composting fermented and naturally rotted artificial composts consisting of straw, hay and dried lawn mowings for the growth of mushrooms are described.

The results of the tests in large pots and boxes on twenty-four different composts are set out and show that previous high temperature fermentation is not absolutely essential for the growth of the cultivated mushroom, although the best results have been obtained from high-temperature composts containing both lime and soil.

The effect of the addition of soil to both fermented and naturally rotted composts is described and discussed.

In conclusion I wish to express my indebtedness to Dr A. C. Fabergé for all the *p*H estimations, to Miss F. M. Durham for testing high-temperature Compost I in the open, and to the laboratory assistant, A. F. Emarton, for the photographs for the plates and for his invaluable help with the preparation and manipulation of the various composts and cultures without which such a number of tests would not have been possible.

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EXPLANATION OF PLATES XV AND XVI

PLATE XV

FERMENTED COMPOSTS

High-temperature composting for 17 *days*

Compost: chopped straw, chopped hay, sulphate of ammonia, lime and superphosphate. 4 months after spawning.

Fig. 1. White market variety (1), first flush, Compost III. Spawn 8 months 10 days old.
Fig. 2. White market variety (1), first flush, Compost IV. Spawn 8 months 10 days old.
Fig. 3. "Honeymoon" variety, first flush, Compost V. Spawn 2 months 1 day old.
Fig. 4. "Honeymoon" variety, second flush, Compost V. Spawn 2 months 1 day old.
Fig. 5. "Honeymoon" variety, third flush, Compost V. Spawn 2 months 1 day old.
Fig. 6. "Honeymoon" variety, first flush, Compost VI. Spawn 2 months 1 day old.
Fig. 7. "Honeymoon" variety, second flush, Compost VI. Spawn 2 months 1 day old.

High-temperature composting for 16 *days*

Compost: chopped straw, dried lawn mowings, sulphate of ammonia and superphosphate. 5 months 10 days after spawning.

Fig. 8. White market variety (2), first flush, Compost IX. Spawn 9 months 21 days old.

High-temperature composting for 31 *days*

Compost: long straw, long hay, chopped straw, chopped hay, lime, sulphate of ammonia and phosphate of potash. 3 months after spawning.

Fig. 9. Bought white commercial spawn, second flush, age of spawn unknown. Compost I.

High-temperature composting for 17 *days*

Compost: chopped straw, chopped hay, sulphate of ammonia and superphosphate. 5 months 11 days after spawning.

Fig. 10. White market variety (3), first flush, Compost III. Spawn 7 months 11 days old.

PLATE XVI

STERILIZED COMPOSTS IN BATTERY JARS

High-temperature composting for 17 *days*

Compost: chopped straw, chopped hay, and sulphate of ammonia.

Fig. 11. White market variety (2), Compost V, after 7 months growth.
Fig. 12. White market variety (2), Compost VI, after 7 months growth.

Natural rotting for 28 *days, without heat*

Compost: chopped straw, chopped hay, and sulphate of ammonia.

Fig. 13. White market variety (3), Compost XVII, after 2 months 3 weeks growth.

Low-temperature composting for 2 months

Compost: chopped straw, chopped hay, and sulphate of ammonia.

Fig. 14. Commercial spawn, after 2 months 2 weeks growth.
Fig. 16. "Honeymoon" variety, after 4 months 2 weeks growth.

Composted stable manure, without lime

Fig. 15. Commercial spawn, after 2 months 2 weeks growth.

Natural rotting for 3 weeks, without heat

Compost: chopped straw, chopped hay, and sulphate of ammonia.

Fig. 17. Wild field mushroom, *Ps. campestris*, Compost XV, after 3 months growth.
Fig. 18. Wild field mushroom, *Ps. campestris*, Compost XVI, after 3 months growth.
Fig. 19. Wild field mushroom, *Ps. campestris*, Compost XVII, after 3 months growth.
Fig. 20. Wild field mushroom, *Ps. campestris*, Compost XVIII, after 3 months growth.

Unfermented, unrotted standard spawn compost

Compost: chopped straw, chopped hay, crushed oats and nutrient solution, without lime or soil.

Fig. 21. Wild field mushroom, *Ps. campestris*, after 2 months growth.
Fig. 22. Wild horse mushroom, *Ps. arvensis*, after 2 months growth.

Pot cultures

Natural rotting for 3 weeks, without heat

Compost: chopped straw, chopped hay, sulphate of ammonia and superphosphate. 6 months onwards after spawning.

Fig. 23. "Honeymoon" variety, first flush, Compost XVII. Spawn 5 months 17 days old.
Fig. 24. "Honeymoon" variety, second flush, Compost XVII. Spawn 5 months 17 days old.
Fig. 25. "Honeymoon" variety, first flush, Compost XVIII. Spawn 5 months 17 days old.

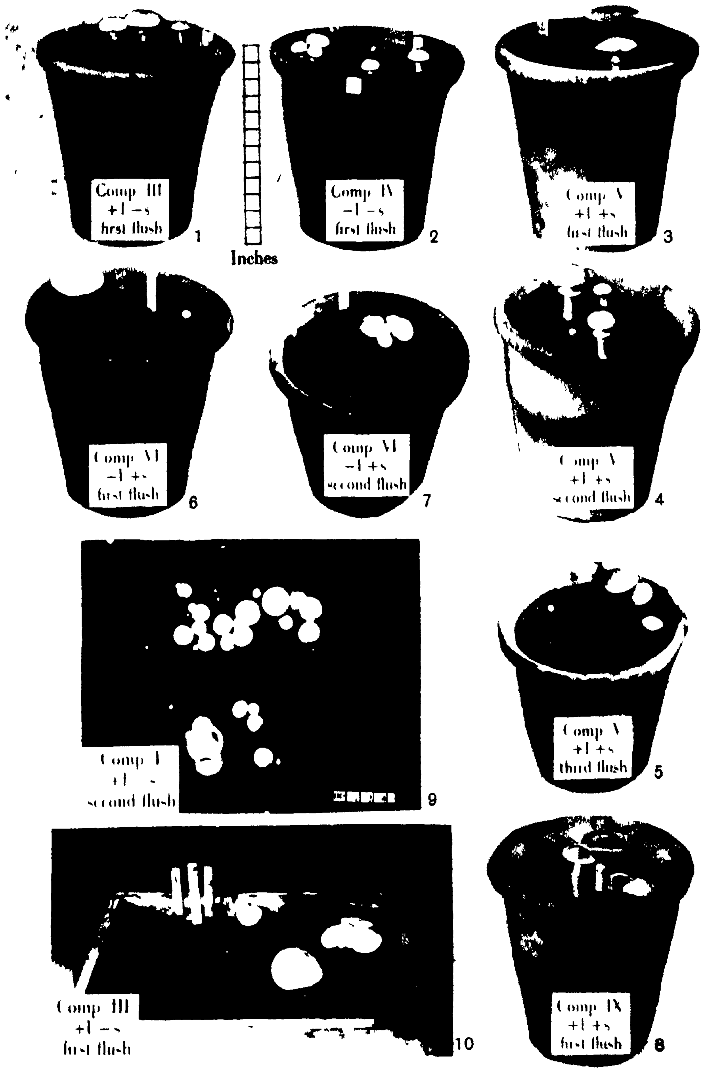
Compost: chopped straw, dried lawn mowings, sulphate of ammonia but *no* superphosphate.

Fig. 26. "Honeymoon" variety, first flush, Compost XX. Spawn 5 months 16 days old.
Fig. 28. "Honeymoon" variety, first flush, Compost XXII. Spawn 5 months 16 days old.

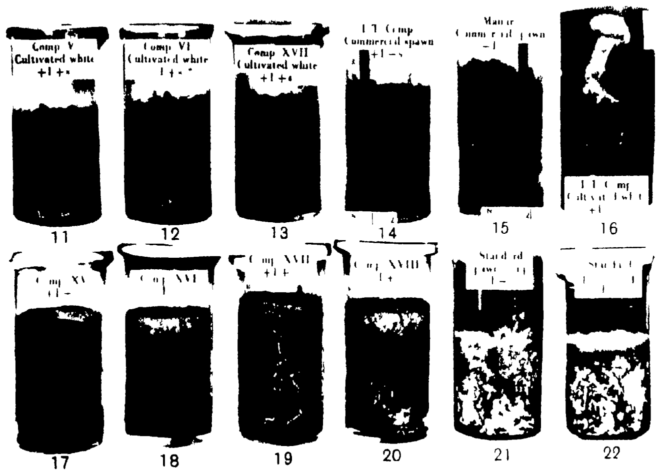
Compost: chopped hay, dried lawn mowings, sulphate of ammonia but *no* superphosphate.

Fig. 27. "Honeymoon" variety, first flush, Compost XXI. Spawn 5 months 16 days old.

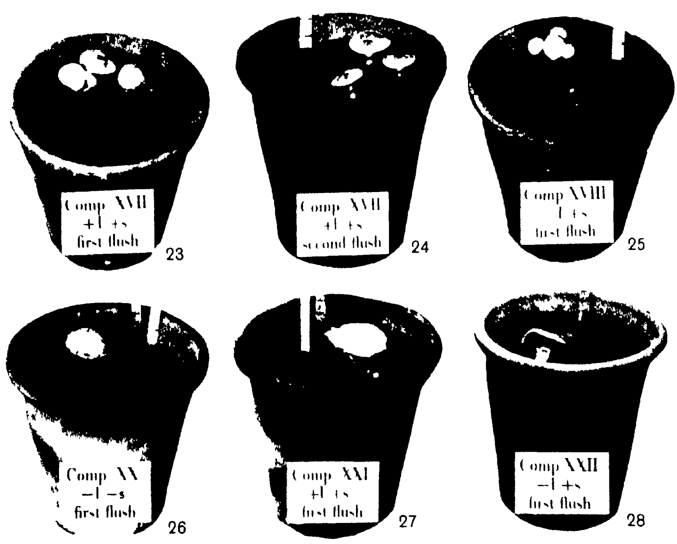
(*Received* 2 *November* 1937)



Fermented high temperature composts.



Spawn on various media



Composts naturally rotted without heat.

FIELD EXPERIMENTS ON THE CONTROL OF WIREWORMS

By W. R. S. LADELL

Entomological Department, Rothamsted Experimental Station

With Appendix

THE INFORMATION SUPPLIED BY THE SAMPLING RESULTS

By W. G. COCHRAN

Statistical Department, Rothamsted Experimental Station

(With 7 Text-figures)

CONTENTS

I. Introduction

King & Glen (1933), reporting progress on wireworm experiments in Saskatchewan, stated "while the necessity for replication is recognized, there seems to exist no basis upon which to determine what might constitute adequacy of replication in respect to the known variability of

wireworm infestation". Further, "preliminary statistical analysis indicates that the methods used for making an annual census of wireworms are reasonably adequate, but final conclusions on this point must await much further study and appraisal". More recently, Jones (1937) compared the figures obtained from sampling units of three sizes and has calculated the errors in sampling at different population densities. The purpose of the present paper is to present the results of three soil fumigation experiments carried out against wireworms on a field scale and using a special field plot technique. It is our object to demonstrate the adequacy of replication required for such types of investigation rather than the value of any particular fumigant.

There are many difficulties in carrying out field trials involving the use of soil fumigants against wireworms. In the first place, wireworms, although easily recognized as such, cannot be readily separated into their correct species. Further, the habits of wireworms as regards the depth at which they are found at different seasons of the year and under different weather conditions are only vaguely understood. This uncertainty is reflected in the different depth and size of the soil samples used by various investigators, such as Roebuck (1924), Miles & Petherbridge (1927), Miles (1932), King (1928, 1929), Lane (1933), Hawkins (1934, 1936 *a*, *b*), Lane & Jones (1936), and Lacroix (1935). Other major difficulties are the choice of a site and the separation of the wireworms from the soil. In connexion with the former, it is sometimes a troublesome task to find exactly when wanted an area which is large enough for the experiment and sufficiently infested with wireworms. The method of separation of the wireworms from the soil must be sufficiently accurate to obtain even the smallest wireworms which, owing to the long life cycle, are found simultaneously with older ones, and sufficiently rapid to allow the samples to be examined before desiccation has caused any diminution in numbers. The effect of the fumigants on the crop must also be examined critically.

Before dealing with the experiments, it is proposed to consider the distribution of wireworms in the field, and then to describe in some detail the methods used in estimating the wireworm population found in the experiments.

While the literature on the prevalence of wireworms, their damage and methods for their control is voluminous, that on sampling technique and properly replicated field experiments designed to show the value of fumigants or other treatments against wireworms is scanty. Reference should be made to Jones (1937), who has recently reviewed the literature

from this point of view, and to Thomas (1930), who reviewed the published research on the control of wireworms.

II. DISTRIBUTION OF WIREWORMS IN THE FIELD

It is commonplace knowledge that wireworms are not uniformly distributed throughout a field, yet care has not often been adequately taken to ascertain their distribution. The reasons for this apparently random distribution are not understood, but no doubt it depends to a large extent on the preference of the wireworms for certain soil conditions and the availability of the requisite food. Thus, it is frequently stated that wireworms in their early instars feed chiefly on humus and only later attack living roots. In the first experiment, the *p*H of all the soil samples was taken with a view to finding out if there was any relation between this factor and the distribution of the wireworms. The *p*H varied considerably, from 5·8 to 6·8 with a mean of 6·3, but no

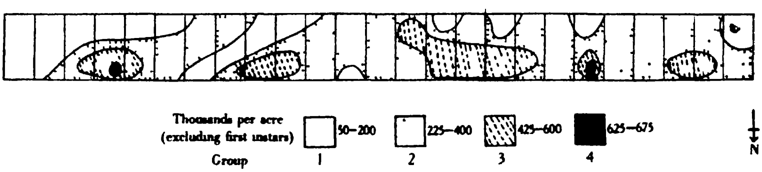


Fig. 1. Preliminary survey of wireworm population in area of old grassland before ploughing up for tests of soil fumigants.

relationship between the reaction of the soil and the number of wireworms was apparent. Although it is outside the scope of the present paper, it may be pointed out that an exact knowledge of the factors underlying the distribution of wireworms in a field might lead to effective preventive measures being taken against an attack. This would be of great value except where old grassland had to be broken up for cultivation. Under such circumstances the use of palliative methods involving soil fumigants must be of major importance.

Fig. 1 illustrates the distribution of wireworms in a strip of land which had not been cultivated for many years and was covered with moss, weeds and grass. This area was chosen for the Knott Wood experiment to be described later. Three random samples, each 9 in. square with a depth of about 5 in., were taken from each half-plot, making a total of 150 samples in all, and the numbers of the wireworms are given in Table I. It will be seen that the distribution is anything but uniform. Emphasis must be laid on the fact that this indicates the distribution of the wireworm population on the date of sampling, March 1935, only.

Table I

Knott Wood experiment. Preliminary survey: before fumigation

No. of wireworms in soil samples 9 × 9 × 5 in.

Serial no. of plot	South half			North half			Totals S	N	S+N	(S−N)
103	0	2	0	2	2	0	2	4	6	2
104	1	0	1	1	2	1	2	4	6	2
105	1	1	0	4	4	10	2	18	20	16
106	2	4	1	6	7	14	7	27	34	20
107	0	5	1	2	3	8	6	13	19	7
108	0	0	4	4	3	4	4	11	15	7
109	5	2	5	3	0	5	12	8	20	4
110	1	5	4	4	9	11	10	24	34	14
111	1	1	5	9	8	1	7	18	25	11
112	2	4	2	5	4	9	8	18	26	10
113	2	8	0	4	5	1	10	10	20	0
114	4	2	3	6	1	1	9	8	17	1
115	4	8	3	5	2	2	15	9	24	6
116	4	5	11	4	4	5	20	13	33	7
117	0	2	2	7	8	3	4	18	22	14
118	6	5	5	8	7	8	16	23	39	7
119	2	0	3	9	3	7	5	19	24	14
120	9	1	3	4	9	10	13	23	36	10
121	5	2	5	4	4	4	12	12	24	0
122	0	4	3	10	11	4	7	25	32	18
123	3	4	3	12	2	2	10	16	26	6
124	3	4	5	0	3	7	12	10	22	2
125	1	5	9	6	6	8	15	20	35	5
126	5	4	2	5	7	6	11	18	29	7
127	4	0	4	5	4	2	8	11	19	3

Standard errors per plot.
Sampling error ± 6·06 or 25 %.
Experimental error ± 6·99 or 29 %.

III. Technique used in assessing the wireworm population by direct counts on soil samples

Soil sampling

It was obvious that extensive soil sampling was essential in order to obtain accurate figures of wireworm infestation. The *size* of the sample used was at first 9 in. square to a depth of about 5 in. Such a sample weighs 20–27 lb. Later, consequent on an improvement in the technique of extracting the wireworms from the samples, a smaller sized sample was used. These later samples were 6 in. cubed and weighed 9–12 lb. The *number* of samples taken in the first experiment was six per plot. Later this number was reduced to four, and in the last experiment (when the size of the plot was only $\frac{1}{200}$ acre) to only two samples per plot.

Where possible, local control was instituted by taking an equal

number of samples in each half-plot. The *time* for sampling was immediately before fumigation and again a few weeks afterwards.

The *position* or *location* of a sample was found by measuring two coordinates from the corner of each plot and was determined beforehand, without any choice on the part of the sampler, by reference to a book of random numbers. This absence of selection on the part of the sampler is essential to obtain a proper estimate of the wireworm population in the area, though this has often been overlooked in previous counts of wireworms. Numbered wooden pegs were used to indicate the positions of the samples in the field.

The *depth* of sample was determined by preliminary sampling which showed that in grasslands the great majority of the wireworms were to be found in the top 4 in. of soil. In order to reduce the amount of soil to be examined, it was decided to use that depth as a standard, and although the figures so obtained would be slightly lower than the truth they would be comparable amongst themselves. In practice it was found that it was impossible to restrict the depth to an accurate 4 in. as the turn-over of the soil sampling tool then used would penetrate to a greater or less depth according to whether the soil was wet and soft, or dry and hard. If the tool was hammered right into the soil so that the top of the sampler was on a level with the ground the depth was 5 in., and it was easier to maintain this as a standard sample. Later it was found that although 5 in. was sufficiently deep for the preliminary sampling on grassland it was not so satisfactory for the second sampling, for which a depth of 6 in. was better, as the soil sample would then include all the partially decomposed grass of the furrow slice and surviving wireworms feeding on the grass roots. Therefore the latest tool was designed to give a sample to a depth of 6 in. when the top was hammered level with the surface of the ground.

Type of soil. The Rothamsted soil is derived from clay-with-flints material and lies over chalk. The irregular presence of flints and the high clay content, up to 30%, makes the task of soil sampling and the subsequent examination a matter of great difficulty, and for these reasons a special tool had to be designed for sampling.

Sampling tool. This is made of $\frac{1}{8}$ in. mild steel plate sharpened and bent to form two sides at right angles with the back, each side being 6 in. long. The top of the plate is turned over externally to give an overlap of 1 in. and the distance from the bottom of this to the cutting edge is 5 in. making a total of 6 in. The tool is hammered into the ground with a "beetle" made of applewood or witch-elm bound with hoop iron. A

straight piece of steel plate is then hammered into the soil across the front of the tool in order to cut the sample. The latter is lifted by slipping a spade underneath from the back while a second operator raises the tool by means of hooks placed in holes at the sides. One operator can, however, take soil samples by the expenditure of a little more time. If possible the soil is kept unbroken to avoid too rapid drying out. On grass or pasture land the grass or weeds are cut close to the ground before the sample is taken.

The samples thus obtained are put into separate canvas or jute sacks together with the numbered peg previously used to indicate the position of the sample in the field and a paper label. Another paper label was fixed externally as an extra precaution, and to assist in arranging the samples for examination.

Preliminary preparation of samples

This process took anything from 15 to 45 min. depending upon the state of the soil. The soil sample was first broken up by tearing it upon a hackle. This is a block of wood with oval nails projecting $1\frac{1}{2}$ in. through it. At one end of this block the nails are set 2 in. apart and at the other end 1 in. The block is fixed to the bench with large "G" clamps. A tray under the bench receives the soil as it falls from the hackle. The wider spaced nails are first used, and then the resulting small sods of soil are broken up still further on the 1 in. spaced nails. Next the grass and weeds are separated as far as possible from the soil which is rubbed through a garden riddle of 3 meshes to an inch. Each portion of soil was examined for wireworms as it came through the riddle. In some of the earlier counts the soil was placed on sheets of brown paper, spread out and searched for any remaining wireworms. The grass and weeds were kept separate and made into parcels for later treatment.

Examination of samples

As has been previously stated, the soil from the samples was at first placed on sheets of brown paper for the final examination. This was found unsatisfactory and recourse was made to a flotation process used in an apparatus devised by the writer (1936) for the separation of insects from the soil. This apparatus could not be used for the experiments described in this paper owing to the large size of the samples.[1] Instead,

[1] A larger machine has been put on the market by Messrs A. Gallenkamp and Co., which will take a 6 in. cube of soil. This machine was used for the most recent sets of samples which, after a preliminary hackling and riddling, were dealt with quickly and efficiently (see p. 348).

the soil was tipped into a 5 gal. oil drum filled to within 3–4 in. of the top with a solution of magnesium sulphate (density 1·11: about 25 % of the commercial salts). Stirring was done by means of wooden stakes for 3 min. After an interval of at least 5 min. the debris was skimmed off the surface by means of a fine mesh strainer (40 meshes to the inch), any adhering to the sides of the drum being removed by the use of a brush. All this debris was washed twice with warm water, squeezed out thoroughly by hand and placed in a tin for further examination later. The stirring and skimming was repeated four or five times until no more material rose to the surface.

The flotation solution may be used over and over again after decanting from the mud and being made up to the necessary strength. The parcels of grass and weeds from the preliminary preparation of the samples were soaked in warm water in a basin for 5 min. and squeezed out, thus freeing them from any adhering soil. This process was repeated and the resulting clean grass and weeds were put into a further tin for later examination. The soil from the grass and weeds was dealt with similarly to the original soil, that is to say, washed through a strainer and any residue was placed in the first tin. Usually the paper label from inside the sample sack was left to mark the oil drum, the wooden label placed in the second tin, i.e. that containing the grass and weeds, while the tie-on paper label from the sack was placed in the first tin, i.e. that containing the debris which was floated.

Examination of floated debris. Naturally most of the wireworms were found in the debris which had been floated up in the solution and this required critical examination. This was done by teasing the debris apart with needles and examining it under a low-power lens mounted on a tripod. Most of the wireworms being alive and active[1] were easily seen; but in some cases where the soil sample had dried out before being dealt with a few dead wireworms occurred and these were not so easily detected.

[1] In the U.S.A., magnesium sulphate is reported by Frings & Frings (1937) to have insecticidal properties, and by Hawkins (1936 *b*) actually to be toxic to the wheat wireworm, whereas I have always found it non-toxic in my laboratory experiments. After immersion all night in the strong solution of magnesium sulphate (25 % of the commercial salts) used in my machine, wireworms were still alive. They were often stiff and rigid but soon recovered after being taken out of the solution. This discrepancy in observations might have been due to a higher temperature in the U.S.A. experiments, to some other important difference in procedure, or merely due to the different species of insect. However, since then, R. C. Smith (1937), of the Kansas Agricultural Experimental Station, has proved that the claims of Frings are unjustified, and that "the small test by Hawkins against the wheat wireworms mentioned by the Frings was apparently not regarded as significant by him, for he drew no practical conclusions from it".

In order to obtain some idea of the different instars being encountered, the wireworms were measured. A cover-slip was used to straighten them before measurement. Six arbitrary groups of $<0{\cdot}5$, 0·6–0·9, 1·0–1·3, 1·4–1·7, 1·8–2·0, and >2 cm. were employed.

Use of Ladell machine (1936) *for separating wireworms from the soil*

Preliminary examination. With a sandy soil such as the Woburn type, the soil sample can be put straight into the machine, but with the clayey soil characteristic of Rothamsted a special technique is required. A further modification is necessary to cope with the mass of undecomposed grass that was present in the soil samples taken from High Field after fumigation.

The soil sample weighing 12–14 lb. is broken up by means of a small garden fork or by the use of the hackle mentioned above. If not too sticky the soil is then passed through a garden riddle. Stones are removed as far as possible. The grass with any adherent soil is soaked in a small quantity of the magnesium sulphate solution (sp. gr. 1·11), sufficient to cover it. The grass is torn apart and rubbed by hand underneath the solution until all the mud is detached, then squeezed and washed in a similar fashion with a further quantity of magnesium sulphate solution. The two lots of muddy solution are mixed together and poured into the machine with the prepared soil.

The grass is washed under running water over a sieve and may be discarded, but the debris on the sieve should be examined for wireworms.

Main process. The main process is as follows: The muddy liquid from the grass is poured into the cylinder with sufficient clean solution to bring the level of the liquid up to the bottom sieve. The soil is then tipped on to the sieve and more solution added until the top sieve is reached. The head is then clamped on, and the cylinder placed in position on the stand, the inlet pipe is connected with the reservoir and more solution allowed to run in. The air pump is connected and switched on, and the stirrer started. After 10 min. the machine is stopped and the liquid allowed to stand for 5 min. All the froth is floated off and then the tap on the cylinder is opened to allow about one-third of its contents to flow away. Afterwards the level of the liquid is brought back to the top of the conical head.

The stirring and bubbling is then continued for a further 2–5 min., the froth being floated off across the surface of the liquid in the tank and collected on a brass sieve with 40 meshes to the inch. The debris on

the sieve is washed twice with water, squeezed out thoroughly by hand and placed in a tin.

This debris is teased apart on a white tray with entomological needles and examined under a low-power lens supported on a tripod. The wireworms are divided into six groups according to their length, as previously stated.

Discussion of the sampling methods used

In an extensive sampling of field plots for wireworms there is great difficulty in examining the large number of soil samples quickly, and this is particularly evident when dealing with the intractable clayey soil of Rothamsted. The first sampling of any experiment should be done immediately before fumigation, but if all the samples are taken on the same day the number is too great to be completed within a reasonable period, and there is danger of serious deterioration of the samples on keeping. As an alternative, the sampling may be spread over a week or two before fumigating, as long as complete blocks are sampled on the same day. This might affect variation between blocks, but not variation within blocks from which the experimental error is derived.

Another difficulty inherent in this type of soil is due to its water-holding capacity. Soil samples taken in the spring are saturated with water and cannot be examined without preliminary drying. For this purpose the sacks containing the sods are hung up on rails with the grass side uppermost, the air circulating round the sacks being sufficient to keep the soil from going sour before it can be examined. Should a reductive phase set in the earlier instars of the wireworms are liable to die. In the cool weather of the spring, the wireworms will remain quiescent in the grass sods for several weeks and none will be lost, but as the weather gets warmer, and especially if the soil dries out too quickly, the wireworms and predators become active and the result is loss by cannibalism or destruction by staphylinid or carabid larvae. These difficulties were all minimized when the size of the soil sample was reduced from 9 to 6 in. square, and the hand examination was superseded by the flotation technique which made it possible to work with the soil while it was still wet.

The second sampling undertaken a few weeks after fumigation was nearly always done in blocks, and the examination of one block completed before the next block was sampled, the reason being that the wireworms were always more active at this later date, the soil drier and not carrying any living grass. Thus the samples had to be examined as

quickly as possible to avoid loss of wireworms. Table IX A is included to show that this extended sampling is not prejudicial to the results. There is no progressive falling off of numbers from the earliest to the latest dates of sampling.

Only in one case was there any serious loss of numbers, and that was with the first sampling of High Field I. The sampling was done very early in the year, at the end of January, and the soil was exceedingly wet. The samples from blocks VII–X, Table VII, had been taken several weeks when a sudden spell of warm weather threatened to dry out the soil too rapidly. One sample from each plot was examined at once and the others moistened. On examining the other three samples from these plots the numbers were found to be almost consistently below those of the first samples, and there was evidence of cannibalism and destruction of wireworms by predators. It was a question of basing the results on one sample only in these plots, or applying a correction to the actual figures obtained. The latter course was the more accurate. The correction was calculated by W. G. Cochran, using the method of least squares, and was applied to the figures from the three moistened samples. Taking as an example block VII "S" and calling the four samples (*a*), (*b*), (*c*), (*d*), (*a*) is the standard sample while (*b*), (*c*) and (*d*) are those that had

Table II

Sampling and experimental errors

Date of sampling	Wire-worms per sq. yd.	Size of sample in.	No. of samples	Fraction of plot sampled %	% error per plot: Sampling	% error per plot: Experi-mental
	Knott Wood. 5 × 5 Latin square. Plots $\frac{1}{60}$ acre					
March 1935						
Before fumigation	65	9 × 9	3 per ¼ plot	0·46	25	29
June 1935						
After fumigation	38	9 × 9	2 per ⅓ plot	0·46	34	50
June 1936						
Residual effect	45*	6 × 6	2 per ½ plot	0·14	48	60
	High Field I. 3 × 10 randomized blocks. Plots $\frac{1}{70}$ acre					
January 1936						
Before fumigation	335	9 × 9	2 per ¼ plot	0·36	19	21
April 1936						
After fumigation	225	6 × 6	2 per ½ plot	0·16	24	34
	High Field II. 6 × 8 randomized blocks. Plots $\frac{1}{110}$ acre					
April 1937						
Before fumigation	277	6 × 6	2 per plot	0·23	37	36
June 1937						
After fumigation	226	6 × 6	2 per plot	0·23	36	31

* Apparent increase due to improvement in technique for separating the wireworms from the

suffered loss. The correction used was $(a)+1{\cdot}763b$ for the first pair of samples and $1{\cdot}763\,(c+d)$ for the second pair:

Actual figures					Corrected figures			
(*a*)	(*b*)	(*c*)	(*d*)	Total	(*a*)	*b′*	*c′*+*d′*	Total
28	11	8	8	55	28	19	28	75

A general summary of the sampling details of the three experiments before and after fumigation are given in Table II, including the population density, amount of sampling done, proportion of total area sampled, with the sampling and experimental errors. This table is referred to by Cochran in his discussion of the experiments from the statistical point of view (see Appendix).

IV. KNOTT WOOD EXPERIMENT

(1) *Lay-out and design of experiment*

For the first fumigation experiment, a strip of land was chosen on the experimental farm between "Pastures" and "Knott Wood" in 1935. This area had not been cultivated for many years and was covered with moss, weeds and grass. Preliminary examination had shown it to be infested with wireworms, mainly *Agriotes sputator* and *A. obscurus*. The land was ploughed and cultivated for sowing with sugar-beet.

The plots were $\frac{1}{60}$ acre in area (40 × 18 ft.) and arranged as a Latin square but with blocks end to end. (For plan see Fig. 2.) There were four treatments and a control, replicated five times:

	Rate per acre cwt.
Chlordinitrobenzene (*N*)	2·0
Chlorpicrin* (*P*)	2·0
Insecticide *K* (*o*- and *p*-dichlorbenzene) (*K*)	5·0
Insecticide *M* (sodium cyanide and anhydrous magnesium sulphate) (*M*)	7·5

* Adsorbed on Kieselguhr.

4 cwt. superphosphate, 2 cwt. muriate of potash and 1 cwt. ammonium sulphate per acre were applied as basal manuring to all plots. The fumigants, diluted with sand, were sprinkled in the bottom of the furrow, using an appropriate measure giving the required quantity for each furrow length. The next furrow slice covered up the fumigant.

This method of application is not ideal but it is useful for experimental purposes with grasslands as it ensures even distribution. The furrow slice should be almost completely inverted and then pressed over by rolling so that the grass roots containing most of the wireworms are in close proximity to the fumigant. In the present instance the rolling was omitted, nevertheless the results were encouraging and suggestive for future work.

(2) *Wireworm counts and conclusions*

Before fumigation.

Late in March 1935, before treatment, three soil samples were taken at random from each half-plot, 150 samples in all, and examined separately for wireworms. Each sample was 9 in. square and taken to a depth of about 5 in.

The number of wireworms obtained in each sample is shown in

Table III

Knott Wood experiment. Wireworm count: after fumigation

Treatment	Serial no. of plot	A	B	C	Totals A	Totals B	Totals C	Totals Per plot
P	103	0,0	1,2	1,2	0	3	3	6
O	104	0,0	0,1	0,2	0	1	2	3
N	105	1,4	7,6	8,3	5	13	11	29
K	106	3,0	0,0	2,3	3	0	5	8
M	107	1,1	4,8	0,3	2	12	3	17
							Block total 63	
M	108	0,0	4,3	1,0	0	7	1	8
K	109	1,4	1,1	3,3	5	2	6	13
O	110	1,1	7,3	2,4	2	10	6	18
N	111	0,2	4,2	2,2	2	6	4	12
P	112	1,0	3,1	9,2	1	4	11	16
							Block total 67	
O	113	2,3	4,3	1,3	4	7	4	15
M	114	1,2	1,2	3,3	4	3	6	13
K	115	0,0	1,4	1,1	0	5	2	7
P	116	2,0	1,1	3,3	2	2	6	10
N	117	1,4	15,3	4,1	5	18	5	28
							Block total 73	
N	118	1,5	4,4	0,0	6	8	0	14
P	119	1,2	1,1	0,6	3	2	6	11
M	120	0,3	4,0	4,2	3	4	6	13
O	121	1,2	3,6	2,8	3	9	10	22
K	122	1,0	1,2	0,3	1	3	3	7
							Block total 67	
K	123	1,3	1,0	2,0	4	1	2	7
N	124	6,4	3,3	5,5	10	6	10	26
P	125	0,6	6,4	2,6	6	10	8	24
M	126	1,1	2,1	7,2	2	3	9	14
O	127	1,2	3,2	5,7	3	5	12	20
							Block total 91	

Per plot

General mean = 14·44.
Experimental error = ±7·185 = 49·8 % G.M.
Sampling error = ±4·87 = 34 % G.M.

Two samples were taken in each third of each plot, the sections being denoted in the table by A, B, C.

Table I, from which it is seen that there is some evidence of a gradient of infestation in the direction of the narrow side of the field. With few exceptions the north, that side away from the wood, was more heavily infested than the south side. The sampling error was 6·06 or 25 % and the experimental error 6·99 or 29 %. Thus the experimental error is almost entirely accounted for by the error in sampling.

After fumigation.

Owing to continuous rain delaying the second sampling, this was commenced only on 24 June, 9 weeks after the application of the fumigants, during very hot weather, and finished on 4 July. Two soil samples were taken in each third of every plot. Samples were taken across the beet rows and included at least one beet plant. In addition one plot in each block was sampled in between the rows. The number of wireworms per sample is shown in Table III.

Table IV shows the number per plot both before and after fumigation and Table IV A gives a comparison between the first and second counts calculated to numbers per square yard. Chlordinitrobenzene showed a reduction in numbers of 15 %, "*M*" and chlorpicrin 45 %, and "*K*"

Table IV

Knott Wood experiment. Wireworm counts: before and after fumigation

Totals of six soil samples ($9 \times 9 \times 5$ in.) per plot. Before above—After below.

Treatments

Block	Chlorpicrin	Chlordinitrobenzene	"*K*"	"*M*"	No fumigant	Block totals
I	6	20	34	19	6	85
	6	29	8	17	3	63
II	26	25	20	15	34	120
	16	12	13	8	18	67
III	33	22	24	17	20	116
	10	28	7	12	16	73
IV	24	39	32	36	24	155
	11	14	7	13	22	67
V	35	22	26	29	19	131
	24	26	7	14	20	91
Treatment	124	128	136	116	103	—
Totals	67	109	42	63	79	—

Standard errors per plot

	Sampling	Experimental
First count	$\pm 6{\cdot}06 = 25\%$	$\pm 7 = 29\%$
Second count	$\pm 4{\cdot}87 = 34\%$	$\pm 7{\cdot}185 = 50\%$

Table IV A

Knott Wood experiment. Summary of results: before and after fumigation

Mean number of wireworms per sq. yd. 5 in. deep

	No fumigant	Chlor-dinitro-benzene	Chlor-picrin	"*M*"	"*K*"	Mean	S.E.
Before fumigation	55	68	66	62	72	65	±8·34
After fumigation	42	58	36	34	22	38	±8·59
Actual difference	13	10	30	28	50	27	
Percentage drop	23	15	45	45	69	42	

69 %. On the untreated controls the density of the population had dropped 23 %, from 55 to 42 per sq. yd., due to seasonal changes, such as a possible movement of the wireworms to a greater depth to escape the heat, early pupation or loss by the activity of birds.

As the numbers of wireworms were so much lower, both the experimental error and sampling error per cent were higher with the second count than they were with the count before treatment. The sampling error was 34 % and the experimental error 50 %. A comparison of the numbers of wireworms at the beet with those found in the soil samples taken in between the rows is given below:

Total of six soil samples

Plot	In row	Between row	Difference −	Difference +
127 (*O*)	20	13	7	
112 (*P*)	16	9	7	
117 (*N*)	28	12	16	
122 (*K*)	7	11		4
107 (*M*)	14	14	0	0
	85	59	−26	
	Total 144			

Thus 59 % of the wireworms were found near the beet and 41 % in between the rows, some slight evidence that the wireworm population has been attracted to the beet. The negative result with the "*K*" plot might be due to the very poor growth of beet coupled with the low numbers of wireworms present.

The analysis of variance on wireworm counts showed that the effects of the treatments on the number of wireworms were not significant, with such a high experimental error (50 %), but that the differences with the "*K*" plots were nearly so. There was no apparent relation between the

numbers of wireworms per plot at the first and second counts after allowing for possible treatment effects.

Figs. 2 and 3 indicate graphically the changes in wireworm population after fumigation.

Effects of fumigants on the crop.

The beet was sown only 5 days after the fumigation of the soil. This resulted in a stunting of the growth of the plants on the plots treated with "*K*" but the other fumigants apparently had no harmful effects. It was important to see how long this deleterious action of "*K*" persisted. On 26 July the soil on the "*K*" plots still smelt strongly of the mixture. For the sake of comparison samples of soil were taken from all the fumigated plots and from the controls, and three pots were filled with the well-mixed soil from each treatment. Oats were planted in

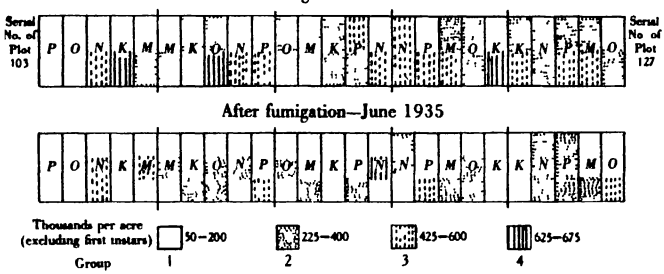


Fig. 2. Knott Wood pastures experiment, wireworm counts.

each pot and made good growth, ultimately reaching maturity. There was no essential difference between the oats grown in the treated soil and those grown in the untreated soil. Thus it would appear that the fumigants applied on 26 April had no longer any toxic effect on the plants 3 months later, even though in the case of "*K*" the soil still smelt strongly of the material.

Table V gives a summary of the yields of roots, tops and sugar obtained. The yield of roots with "*M*" was significantly higher than that with chlorpicrin or chlordinitrobenzene, the latter yields not being significantly different from the yield with no fumigant. The response to "*M*" may be an effect of nitrogen, the dressing being equivalent to 87 lb. nitrogen per acre.

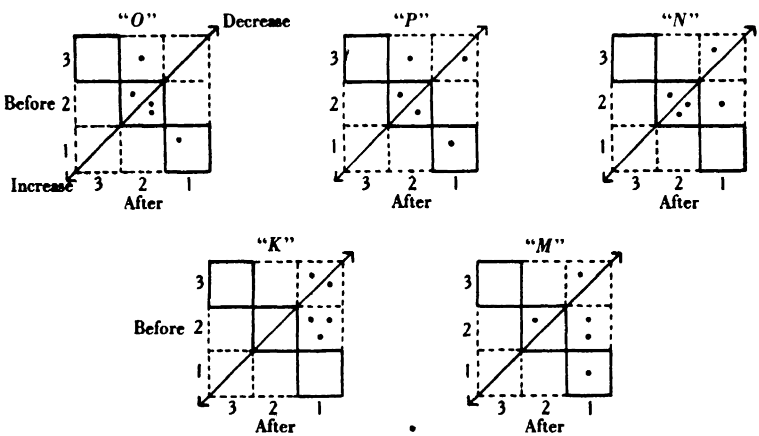
"*M*" and chlordinitrobenzene significantly increased the yields of tops, the increases not being significantly different. There were no significant effects on sugar percentage, apart from the reduction due to "*K*".

Table V

Knott Wood experiment. Beet crop. Summary of results

	No fumigant	Chlordi-nitroben-zene	Chlor-picrin	"*M*"	"*K*"	Mean	Standard error (treatment means)
Roots (washed), tons per acre	8·90	9·54	9·11	10·91	3·64	8·42	±0·426*
Tops, tons per acre	10·44	12·57	10·22	13·67	5·04	10·39	±0·519*
Sugar %	16·16	15·92	16·16	16·24	15·22	15·94	±0·130
Total sugar, cwt. per acre	28·8	30·4	29·4	35·4	11·1	27·0	—
Plant number, thousands per acre	28·6	29·0	29·4	30·0	26·3	28·7	±0·528

* These standard errors are not applicable to the "*K*" treatment.



Group	1	2	3
Thousands per acre	50–200	225–400	425–600

Fig. 3. Knott Wood pastures experiment. Change in distribution of wireworm population before and after fumigation.

The figure indicates graphically the changes in population estimated from samples taken before and after fumigation. The vertical scale is the population group before fumigation, and the horizontal scale the group after fumigation.

The heavily lined squares are areas of no change. Each dot represents one plot. Dots above the heavily lined squares represent plots which showed a decrease in population after fumigation, and dots below them represent plots which showed an increase.

Thus with "*O*", four plots remain in the same population group and one plot changes from group 3 (425–600 thousands) to group 2 (225–400 thousands), whereas with "*K*" three plots change from group 2 before fumigation to group 1 (50–200 thousands) afterwards, and two plots from group 3 to group 1.

The average change, as a percentage on the original count, is as follows: "*O*" −23, "*P*" −45, "*N*" −15, "*K*" −69, "*M*" −45.

"*K*" gave low yields and a low sugar percentage. There is no doubt that this was because it was applied too near the sowing date.

No relation was found between the yields of roots and the numbers of wireworms at the second count, after eliminating treatment effects.

Residual effects.

No further treatment was given in 1936, but barley was drilled on 20 March. Four soil samples (6×6 in.) were taken per plot, two samples in each half-plot, in June 1936, one year after the second soil sampling. The total number of wireworms obtained from the five plots for each treatment was as follows:

"*O*"	29
Chlorpicrin	23
Chlordinitrobenzene	35
"*K*"	6
"*M*"	31

The figures for separate plots are given in Table VI. Thus the fewest wireworms were again found on the "*K*" plots.

Table VI

Knott Wood experiment. Residual effect

Number of wireworms per plot June 1936. Total of 4 samples ($6 \times 6 \times 5$ in.)

					Rows
P 3	*O* 2	*N* 5	*K* 1	*M* 4	15
M 6	*K* 0	*O* 6	*M* 4	*P* 4	20
O 4	*M* 9	*K* 1	*P* 6	*N* 5	25
N 17	*P* 8	*M* 8	*O* 9	*K* 0	42
K 4	*N* 4	*P* 2	*M* 4	*O* 8	22
Columns 34	23	22	24	21	124

G.M. $= 4 \cdot 96$. Sampling error 48%. Experimental error $= \pm 2 \cdot 976 = 60\%$.

The mean stand of plants per 4 m. length was counted in May and varied from 140 on the untreated plots to 197 on the plots which had been treated with "*K*" in 1935. Thus the plots with the biggest reduction of the wireworm population gave the best stand of barley. However, these differences in the plant numbers were not reflected in the final yield of grain. The controls yielded an average of 18·1 cwt. per acre, and

the treated plots varied from 19·5 cwt. with "*K*" to 21·1 cwt. with chlordinitrobenzene. Either the general infestation was not high enough to cause material damage or the plants had made extra growth to compensate for damage done. The crop of barley was a moderately good one for this season which was not particularly favourable to cereals. It is suggested that the high yield from the chlordinitrobenzene plots might be due to delayed nitrogen effect, the chlorine having inhibited the breakdown of the compound by bacterial action in the year of application.

Residual effect, summary of results

Mean number of wireworms per sq. yd. to 5 in. deep

No fumigant	Chlordinitro-benzene	Chlorpicrin	"*M*"	"*K*"	Mean	S.E.
52	63	41	56	11	45	±12

The mean number of wireworms found is 45 per sq. yd. compared with 38 per sq. yd. in June 1935. This increase is more apparent than real and is attributed to the improvement in the technique for separating the wireworms, whereby the last count includes the earlier instars which had been missed in the first and second counts.

V. High Field experiment I

(1) *Lay-out and design of experiment*

For this experiment an area of old park land was selected in High Field, Rothamsted, to break up for a crop of barley. The object of the experiment was to try out "*S*" and chlorpicrin, these being two of the most promising fumigants from the previous trial. In this experiment, it was decided to increase the replication from five to ten and reduce the samples from six to four. The plots were $\frac{1}{70}$ acre in area (25·25 × 25 ft.) and arranged in ten randomized blocks of three plots each. (For plan see Fig. 4.)

There were two treatments and a control.

	Rate per acre
(*S*)*	800 lb.
Chlorpicrin (*C*)†	245 lb.
No fumigant	

* A similar mixture to "*K*" used in Experiment 1 but containing a higher proportion of *o*-dichlorbenzene.

† In the form of pellets containing 25% chlorpicrin.

The fumigants were applied in the same fashion as in the Knott Wood Experiment by sprinkling an appropriate quantity at the bottom of each plough furrow.

(2) *Wireworm counts and conclusions*

Before fumigation.

At the end of January 1936 two soil samples (9 × 9 × 5 in.) were taken from each half-plot, a total of 120 samples. The numbers of wireworms found in each sample are shown in Table VII. The total number of wireworms found was 2516, an average of 21 per sample. This is five times the number found in the preliminary examination in the Knott Wood experiment, the difference being mostly due to an actually higher infestation, but partly to the improvement in the technique which made it possible to discover the smaller wireworms which were missed by the crude methods used at first.

Table VII

High Field I experiment. Wireworm counts: before fumigation

Two soil samples per half-plot. Size of samples: 9 × 9 × 5 in.

Plot totals thus 99

Plots 1–15

Block	Chlorpicrin	"*S*"	No fumigant	Block totals
I	20 6 74 36 12	21 18 78 18 21	36 15 99 30 18	251
II	21 13 71 23 14	20 19 79 20 20	14 12 72 28 18	222
III	23 8 65 21 13	20 34 96 18 24	10 7 60 13 30	221
IV	18 20 95 27 30	32 30 92 20 10	31 6 78 25 16	265
V	28 15 84 20 21	58 39 137 21 19	19 25 73 17 12	294
Treatment totals	389	482	382	1253

G.M. = 83·87. Sampling error = 18·7 %. Experimental error ± 17·75 = 21·2 %.

Table VII (*cont.*)

Plots 16–30

Block	Chlorpicrin			"*S*"			No fumigant			Block totals
VI	25		16	34		12	14		26	
		95			105			69		269
	36		18	24		35	18		11	
VII	13		19	28		19	24		28	
		66			75			100		241
	9		25	14		14	18		30	
VIII	21		7	16		19	22		7	
		54			118			82		254
	7		19	18		65	26		27	
IX	11		21	19		26	33		33	
		66			86			105		257
	16		18	20		21	18		21	
X	12		16	15		21	4		32	
		74			98			69		241
	26		20	48		14	26		7	
Treatment totals		355			482			425		1262

The larger number of wireworms and greater replication gave lower sampling and experimental errors per cent: 18·7 and 21·2 respectively.

After fumigation.

Owing to various adverse circumstances, the interval between the fumigation and the second sampling was again longer than desirable, being a minimum of 12 weeks.

The second sampling commenced on 30 April and extended to 16 May, two blocks generally being sampled in a day. The size of the samples was $6 \times 6 \times 5$ in. and two were taken in each half-plot.

Table VIII gives the numbers of wireworms found in each sample. The block totals varied from 51 to 113 with a mean of 75, a block total representing the number of wireworms found in twelve soil samples.

The treatment totals for ten plots are as follows: "*S*", 165, chlorpicrin, 221 and no fumigant, 363. The means are: "*S*", 16·5, chlorpicrin, 22·1 and no fumigant, 36·3, with a general mean of 24·97.

Table IX compares the plot totals before and after fumigation. Table IX A shows that the extension of the sampling period over 6 weeks has not affected the results.

Table IX B, which summarizes the wireworm counts before and after fumigation, shows that the average density of population on the untreated plots has not materially changed, being 323 per sq. yd. before fumigation and 327 after fumigation, an increase of only 1·2 %. This is in contrast to the Knott Wood experiment where a drop of 23 % was found. The period covered by the two counts on Knott Wood was March–July, whereas on High Field I it was January–May. A possible explanation is a downward movement of the wireworms in June to below the depth of sampling, as mentioned above, and loss by pupation. "*S*" has reduced the population by 61·2 % and chlorpicrin by 33·2 %. Both these

Table VIII

High Field I experiment. Wireworm count: after fumigation

Two samples per half-plot. Size of samples: 6 × 6 × 5 in.
Total per plot in central square

Plots 1–15

Blocks	Chlorpicrin	"*S*"	"*O*"	Block totals
I	5 4 $\boxed{16}$ 5 2	5 5 $\boxed{13}$ 1 2	12 20 $\boxed{48}$ 8 8	77
II	0 9 $\boxed{15}$ 3 3	6 4 $\boxed{19}$ 5 4	7 4 $\boxed{20}$ 4 5	54
III	4 4 $\boxed{20}$ 3 9	2 9 $\boxed{21}$ 3 7	9 6 $\boxed{33}$ 7 11	74
IV	7 3 $\boxed{27}$ 5 12	6 4 $\boxed{22}$ 8 4	12 22 $\boxed{64}$ 17 13	113
V	4 9 $\boxed{27}$ 8 6	2 9 $\boxed{21}$ 7 3	7 8 $\boxed{29}$ 5 9	77
Treatment totals	105	96	194	395

G.M. = 24·97. Sampling error = 23·8 %. Experimental error ± 8·43 = 33·8 %.

Table VIII (*cont.*)

Plots 16–30

Blocks	Chlorpicrin	"*S*"	"*O*"	Block totals
VI	8, 15; 5, 4 $\boxed{32}$	1, 5; 0, 2 $\boxed{8}$	14, 7; 16, 8 $\boxed{45}$	85
VII	4, 4; 3, 3 $\boxed{14}$	5, 4; 6, 6 $\boxed{21}$	7, 9; 2, 10 $\boxed{28}$	63
VIII	3, 2; 13, 4 $\boxed{22}$	6, 3; 3, 2 $\boxed{14}$	4, 5; 18, 8 $\boxed{35}$	71
IX	11, 12; 3, 5 $\boxed{31}$	3, 5; 5, 5 $\boxed{18}$	10, 12; 8, 5 $\boxed{35}$	84
X	7, 6; 3, 1 $\boxed{17}$	4, 4; 0, 0 $\boxed{8}$	6, 9; 3, 8 $\boxed{26}$	51
Treatment totals	116	69	169	354
Grand totals	221	165	363	749

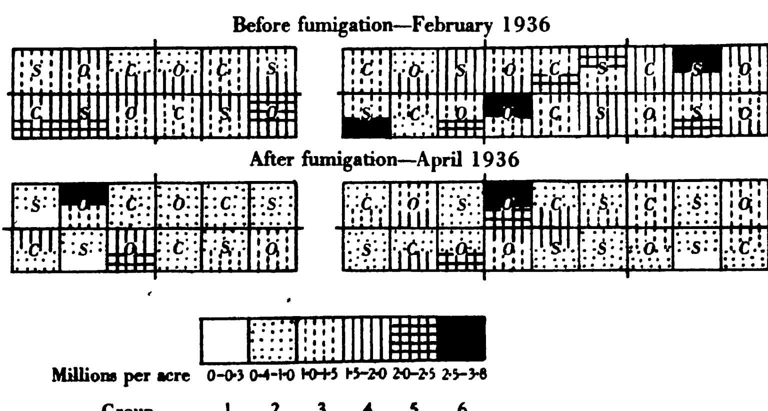


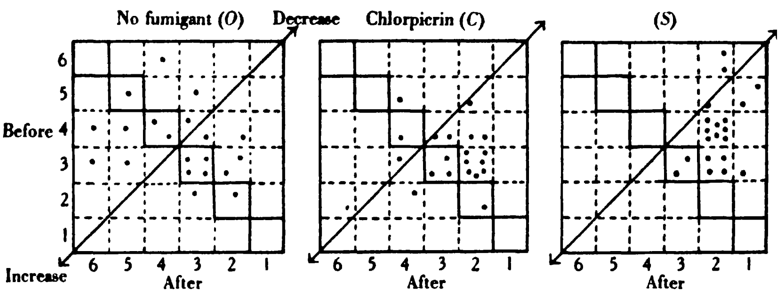
Fig. 4. High Field I, wireworm counts. The variation in shading indicates the population densities per half-plot.

differences are significant. Figs. 4 and 5 show graphically the changes after fumigation.

The sampling error was 23·8 % of the general mean, not much greater than that of the sampling before fumigation in spite of the smaller sampling unit. The experimental error was 33·8 %, higher than that of the preliminary sampling. As in the first experiment, there was no relation between the numbers of wireworm per plot at the first and second counts after allowing for treatment effects. As far as the wireworm counts are concerned this experiment was quite satisfactory but it was not so successful with the crop.

Effect of fumigants on crop.

Soon after the barley was planted it was noticed that the birds were very busy on the plots and paper streamers were fixed in order to frighten



Group	1	2	3	4	5	6
Millions per acre	0–0·3	0·4–1	1–1·5	1·5–2	2–2·5	2·5–3·8

Fig. 5. High Field I. Change in distribution of wireworm population before and after fumigation.

The figure indicates graphically the changes in population estimated from samples taken before and after fumigation. The vertical scale is the population group before fumigation, and the horizontal scale the group after fumigation. The heavily lined squares are areas of no change. Each dot represents one half-plot. Dots above the heavily lined squares represent plots which showed a decrease in population after fumigation, and dots below them represent plots which showed an increase.

The diagram clearly shows the big reduction in population brought about by the use of chlorpicrin and the still bigger reduction by the fumigant "*S*". With no fumigant there are eight half-plots that show no change in the population group, five increases and seven small decreases. With chlorpicrin there are five half-plots showing no change, two small increases, and twelve decreases, one of which is from group 5 (2–2·5 million) to group 2 (0·4–1 million) and two from group 4 (1·5–2 million) also to group 2. With "*S*" only two half-plots remain in the same group, there are eighteen decreases, two of which are from group 6 (2·5–3·8 million) to group 2, two from group 5 to group 1 (below 0·3 million), one from group 5 to group 2, and eight from group 4 to group 2. The average change as a percentage on the count before fumigation is "No fumigant" +1·2, Chlorpicrin −33·2, "*S*" −62·2.

Table IX

High Field I experiment. Wireworm counts: before and after fumigation

Plot totals: first count above, second count below.*
Total of four samples (9 × 9 × 5 in.) per plot

Plots 1–15

Blocks	Chlorpicrin	"S"	No fumigant	Block totals
I	74 36	78 29	99 109	251 174
II	71 34	79 43	72 45	222 122
III	65 45	96 48	60 75	221 168
IV	95 61	92 50	78 144	265 255
V	84 61	137 48	73 64	294 173
Treatment totals:				
Before	389	482	382	
After	237	218	437	

Plots 16–30

Blocks	Chlorpicrin	"S"	No fumigant	Block totals
VI	95 72	105 18	69 112	269 202
VII	66 32	76 48	100 64	242 144
VIII	54 50	118 33	82 78	254 161
IX	66 70	86 40	105 79	257 189
X	74 39	98 18	69 59	241 116
Treatment totals:				
Before	355	483	425	
After	263	157	393	

* Actual size of sample at second count 6 × 6 × 5 in. Figures calculated to 9 × 9 in. for comparison with first count.

Table IXA

High Field I experiment. Wireworm counts: after fumigation

Dates of sampling and block totals

Plots no.	Date of sampling	Wireworms in blocks of three plots
1, 2, 3	30 April	77
4, 5, 6	2 May	54
19, 20, 21	2 „	63
7, 8, 9	8 „	74
16, 17, 18	8 „	85
10, 11, 12	12 „	113
25, 26, 27	12 „	84
22, 23, 24	14 „	71
13, 14, 15	16 „	77
28, 29, 30	16 „	51

Table IXB

High Field I experiment. Summary of results: before and after fumigation

Mean no. of wireworms per sq. yd. (5 in. deep)

	No fumigant	Chlor-picrin	"*S*"	Mean	S.E.
Before fumigation	323	298	386	335	±41
After fumigation	327	199	150	225	±44
Difference:					
Actual	+4	−99	−236	−110	
% on original count	1·2	−33·2	−62·2	−32·8	

them away. There is no doubt that they removed a large number of the germinating seedlings and probably some of the wireworms as well. It was not possible to observe whether they concentrated on some plots more than others, but fumigants used may have had a deterrent effect on the birds which would then tend to gather more from the untreated plots.

A stand count was taken on 13 May. The mean number of plants per 4 m. length varied from 52 on the "*S*" plots to 85 on the chlorpicrin plots, with the controls occupying an intermediate position with 68. The reduction in stand on the "*S*" plots was not quite significant but the increase in the stand on the chlorpicrin plots was significant.

There was an interval of 6 weeks between the application of the fumigants and drilling the barley. Evidently this interval was not long enough to dissipate the phytocidal action of the "*S*" with the large quantity used, 8 cwt. compared with 5 cwt. used in Knott Wood. In view of the poor stand it was decided that no further information would be obtained by allowing the crop to mature, so the land was harrowed

and sugar-beet planted. Before this, the second soil sampling had been completed. The barley should have been grubbed out immediately, but unfortunately a spell of very wet weather intervened and, before the barley was pulled, it had grown to the flowering stage and choked out the sugar-beet. Thus the experiment finished with no crop at all.

Residual effect.

No further treatment was given in 1937 but barley was sown once again. A third wireworm count was not made as it was considered that the time available would be better employed by starting a new experiment.

Stand count. The stand of barley was poor and very similar to that of 1936, the means varying from 55 plants per 4 m. on the untreated plots to 66 on the "*S*" plots and 69 on the chlorpicrin plots. The average increase on the fumigated plots was significant, but there was no indication of any difference between the treatments.

Yields. The final yield of barley was very poor; this may have been due partly to the fact that no fertilizers were used and partly to damage by wireworms. The controls yielded an average of 10·1 cwt.: "*S*", 9·6 cwt. and chlorpicrin, 12·3 cwt. of grain per acre. The response to chlorpicrin was significant. This increase may be a result of the reduction in the number of wireworms, but on the other hand the possibility of a delayed nitrogen effect due to the breakdown of the chlorpicrin cannot be ignored.

VI. High Field experiment II

(1) *Lay-out and design of experiment*

The object of the experiment was to test the effect of various materials, frequently recommended as efficacious, on wireworm infestation in old grassland which was ploughed up and summer fallowed for winter corn.

There were 48 plots of $\frac{1}{200}$ acre in randomized blocks.

The following treatments were given:

No treatment.

Lime: 34 cwt. per acre.

Tar and lime (33 % tar): 51 cwt. per acre.

Ammonium carbonate: 70 lb. per acre (equivalent in nitrogen content to the tar).

Superphosphate: 6 cwt. per acre.

Naphthalene (crude): 10 cwt. per acre.

Fig. 6 shows the plan for the experiment.

The fumigants were applied at the bottom of the plough furrows on 9 April 1937. On 11 April the land was rolled to complete the inversion of the furrow slice and consolidate the soil.

(2) *Wireworm counts and conclusions*

Soil sampling before treatment.

Two soil samples (6 × 6 × 6 in.) per plot were taken on 23 and 24 March, a total of 96 samples, 16 days before fumigants were applied. The soil was very wet after weeks of persistent rainfall which made the subsequent examination very troublesome.

The number of wireworms per soil sample and per plot are shown in Table X. The figures range from 1 to 19 per sample with an average of 7·2, and from 4 to 32 per plot with an average of 15·38. The sampling error and experimental error are practically the same, 36·8 and 35·6 %

Table X

High Field II experiment. Wireworm count: before treatment

Two soil samples per plot (6 × 6 × 6 in.). Plot totals in squares

Blocks	Treatments																		Block totals
	O			Lime			Tar + lime			Am. carb.			Super.			Naphth.			
I	13	$\boxed{20}$	7	2	$\boxed{7}$	5	15	$\boxed{20}$	5	9	$\boxed{21}$	12	7	$\boxed{16}$	9	10	$\boxed{22}$	12	106
II	13	$\boxed{20}$	7	9	$\boxed{14}$	5	10	$\boxed{21}$	11	6	$\boxed{16}$	10	7	$\boxed{18}$	11	5	$\boxed{12}$	7	101
III	14	$\boxed{25}$	11	7	$\boxed{19}$	12	5	$\boxed{9}$	4	3	$\boxed{7}$	4	7	$\boxed{8}$	1	11	$\boxed{20}$	9	88
IV	8	$\boxed{9}$	1	5	$\boxed{8}$	3	5	$\boxed{21}$	16	12	$\boxed{18}$	6	8	$\boxed{10}$	2	6	$\boxed{13}$	7	79
V	2	$\boxed{4}$	2	4	$\boxed{13}$	9	3	$\boxed{10}$	7	7	$\boxed{8}$	1	7	$\boxed{13}$	6	6	$\boxed{10}$	4	58
VI	7	$\boxed{18}$	11	11	$\boxed{13}$	2	7	$\boxed{16}$	9	8	$\boxed{13}$	5	4	$\boxed{16}$	12	4	$\boxed{22}$	18	98
VII	15	$\boxed{19}$	4	8	$\boxed{12}$	4	9	$\boxed{16}$	7	9	$\boxed{14}$	5	4	$\boxed{7}$	3	13	$\boxed{32}$	19	100
VIII	11	$\boxed{23}$	12	3	$\boxed{14}$	11	10	$\boxed{16}$	6	10	$\boxed{16}$	6	12	$\boxed{16}$	4	15	$\boxed{23}$	8	108
Treatment totals		138			100			129			113			104			154		738

G.M. = 15·38. Sampling error ± 5·65 = 36·8 %. Experimental error ± 5·48 = 35·6 %.

respectively, i.e. the inter-plot variation is no more than the variation between individual soil samples. The two soil samples only represented 0·23 % of the total area of the plot, this small proportion being a contributory cause of the high sampling error.

The average density of the wireworm population was 277 per sq. yd., which is considerably less than the 335 per sq. yd. found on High Field in January 1936.

Soil sampling after treatment.

Again two 6 in. samples were taken from each plot. The sampling and examination commenced some weeks after the application of the treatments extending from 1 to 24 June. Details of the numbers of wireworms found are shown in Table XI and a comparison of the counts before and after treatment are shown in Tables XII and XII A. The figures for individual samples ranged from 2 to 16 with a mean of 6·25. The figures per plot were 5–23 with a mean of 12·5. The sampling error

Table XI

High Field II experiment. Wireworm count: after treatment

Two soil samples per plot (6 × 6 × 6 in.). Plot totals in squares

Block	Treatments						Block totals
	No treatment	Lime	Tar + lime	Am. carb.	Super.	Naphth.	
I	8 $\boxed{18}$ 10	8 $\boxed{19}$ 11	10 $\boxed{14}$ 4	10 $\boxed{17}$ 7	9 $\boxed{16}$ 7	3 $\boxed{10}$ 7	94
II	4 $\boxed{18}$ 14	5 $\boxed{8}$ 3	8 $\boxed{13}$ 5	5 $\boxed{7}$ 2	5 $\boxed{11}$ 6	2 $\boxed{6}$ 4	63
III	14 $\boxed{21}$ 7	8 $\boxed{13}$ 5	4 $\boxed{11}$ 7	9 $\boxed{15}$ 6	8 $\boxed{12}$ 4	3 $\boxed{5}$ 2	77
IV	9 $\boxed{20}$ 11	7 $\boxed{15}$ 8	2 $\boxed{10}$ 8	8 $\boxed{12}$ 4	16 $\boxed{20}$ 4	3 $\boxed{7}$ 4	84
V	10 $\boxed{15}$ 5	5 $\boxed{9}$ 4	5 $\boxed{9}$ 4	3 $\boxed{9}$ 6	3 $\boxed{11}$ 8	3 $\boxed{5}$ 2	58
VI	5 $\boxed{8}$ 3	16 $\boxed{23}$ 7	4 $\boxed{8}$ 4	3 $\boxed{7}$ 4	7 $\boxed{11}$ 4	4 $\boxed{7}$ 3	64
VII	5 $\boxed{16}$ 11	7 $\boxed{15}$ 8	3 $\boxed{6}$ 3	2 $\boxed{15}$ 13	4 $\boxed{15}$ 11	7 $\boxed{11}$ 4	78
VIII	6 $\boxed{10}$ 4	10 $\boxed{15}$ 5	9 $\boxed{21}$ 12	5 $\boxed{8}$ 3	11 $\boxed{17}$ 6	3 $\boxed{11}$ 8	82
Treatment totals	126	117	92	90	113	62	600

G.M. = 12·5. Sampling error ± 4·45 = 35·6 %. Experimental error ± 3·90 = 31·2 %.

was 35·6 %, accounting for the whole of the experimental error which is 31·2 %.

Table XII

High Field II experiment. Wireworm counts: before and after treatment

Total of two soil samples (6 × 6 × 6 in.) per plot. First count above, second count below

Treatments

Blocks	No treat-ment	Lime	Tar + lime	Am. carb.	Super.	Naphth.	Block totals	Diff.
I	20	7	20	21	16	22	106	
	18	19	14	17	16	10	94	−12
II	20	14	21	16	18	12	101	
	18	8	13	7	11	6	63	−38
III	25	19	9	7	8	20	88	
	21	13	11	15	12	5	77	−11
IV	9	8	21	18	10	13	79	
	20	15	10	12	20	7	84	+ 5
V	4	13	10	8	13	10	58	
	15	9	9	9	11	5	58	0
VI	18	13	16	13	16	22	98	
	8	23	8	7	11	7	64	−34
VII	19	12	16	14	7	32	100	
	16	15	6	15	15	11	78	−22
VIII	23	14	16	16	16	23	108	
	10	15	21	8	17	11	82	−26
Treatment totals:								
Before	138	100	129	113	104	154		
After	126	117	92	90	113	62		
Diff.	−12	+17	−37	−43	+9	−92		

Table XII A

High Field II experiment. Summary of results: before and after treatment

Mean no. of wireworms per sq. yd. (6 in. deep)

	No treat-ment	Lime	Tar + lime	Am. carb.	Super.	Naphth.	Mean	S.E.
Before treatment	310	225	290	254	234	346	277	±40
After treatment	283	263	207	202	254	139	225	±29
Difference:								
Actual	−27	+38	−83	−52	+20	−207	−52	
% on original count	− 8·7	+17	−28·6	−20·4	+ 8·6	− 59·8	−18·8	

It will be seen from the treatment totals given in Table XI that all the treated plots have fewer wireworms than the untreated plots. *Taking*

the "no treatment" number as a datum and calculating the differences as a percentage on this, the following figures are obtained:

		Differences	
		Actual	Percentage
No treatment	126	—	—
Lime	117	- 9	- 7·2
Superphosphate	113	-13	-10·3
Tar and lime	92	-34	-27
Ammonium carbonate	90	-36	-28·6
Naphthalene	62	-64	-50·5

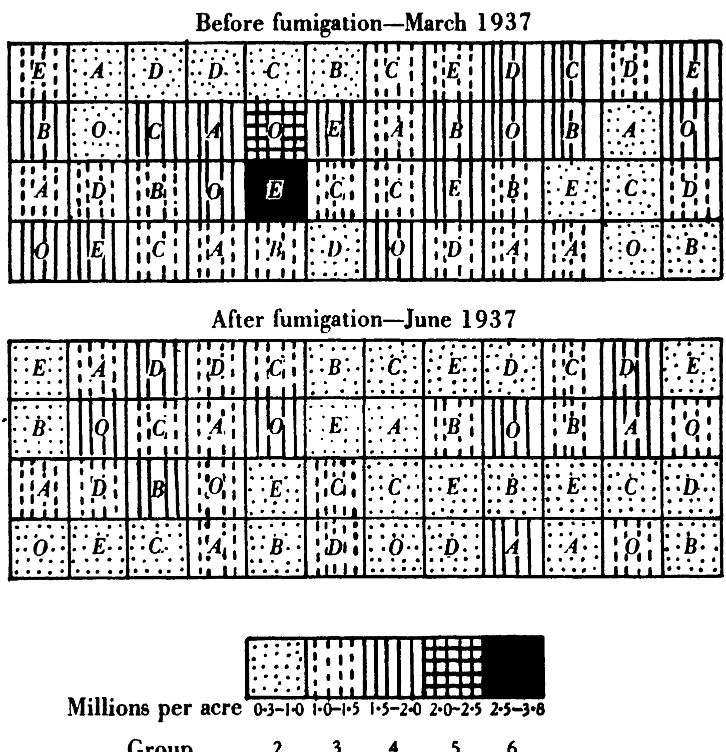


Fig. 6. High Field II, wireworm count. The variation in shading indicates the population densities per plot.

The analysis of variance showed that these differences were significant in the case of tar and lime, ammonium carbonate and naphthalene, but the differences between the latter and the two former is not quite

significant. Figs. 6 and 7 indicate graphically the changes after treatment.

Comparing the counts before and after treatment (Table XII A).

It is seen that the mean density of the wireworm population had fallen from 277 to 225 per sq. yd. The untreated plots have dropped by

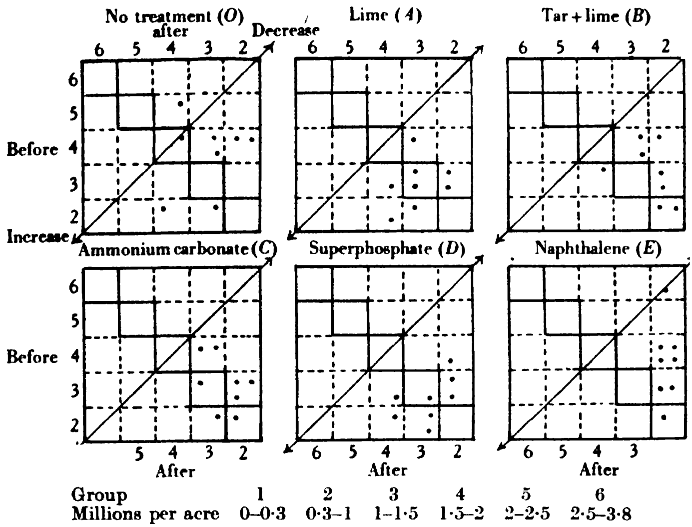


Fig. 7. High Field II. Change in distribution of wireworm population, before and after treatment.

The figure indicates graphically the change in population estimated from samples taken before and after fumigation. The vertical scale is the population group before fumigation and the horizontal scale the group after fumigation. The heavily lined squares are areas of no change. Each dot represents one plot. Dots above the heavily lined squares represent plots which showed a decrease in population after fumigation, and dots below them represent plots which showed an increase. For example, the untreated plots show five decreases, one unchanged and two increases, whereas the naphthalene plots show seven decreases of which one is from group 6 (2·5–3·8 million) to group 2 (0·3–1 million) and four are from group 4 (1·5–2 million) also to group 2.

The average change, as a percentage on the count before fumigation is as follows: no treatment −8·7, lime +17, tar + lime −28·6, ammonium carbonate −20·4, superphosphate +8·6, naphthalene −59·8.

8·7 %, ammonium carbonate by 20·4 %, tar and lime by 28·6 %, and naphthalene by 59·8 %, whereas the limed plots have increased by 17 % and superphosphate by 8·6 %. As far as the significant treatments are concerned these results are not materially different from the conclusions obtained from the consideration of the second count alone. The apparent

increases on the lime and superphosphate plots are small and of no real significance. The failure of lime to reduce the number of wireworms is interesting in view of its frequent recommendation amongst farmers. Superphosphate has often been mentioned as a dressing for land infested with wireworms, but a direct toxic effect would be unlikely. Any value it might have would depend on its stimulating action on root growth, thus encouraging the crop to grow away quickly from the attack. Although the reduction in the wireworm population produced by the fumigants (even the 59·8 % with naphthalene) is too low to be of much economic value, yet the figures are useful as indicating possible means of control. More adequate control could be expected, given a finer physical condition of the fumigant, and a better method of application resulting in a more complete distribution throughout the soil mass. It will be necessary to find out how far the reduction in population by any treatment is due to a repellent action and how far it is actually lethal and also whether any repellent action is lateral or downwards. Experiments are being made to throw light on these points.

The reduction in numbers of 8·7 % between the first and second samplings on the control plots is less than that found in the Knott Wood experiment and more than on High Field I, thus:

	Time of sampling		Average difference in controls
	1st	2nd	%
Knott Wood	March	July	−23
High Field I	January	May	+ 1·2
High Field II	March	June	− 8·7

A number of the wireworms found in the second sampling of High Field II were in process of moulting, and some were actually pupating but not enough to affect the percentage drop. As already suggested, the reduction when the second sampling was done in June and the still bigger one when the sampling was done in July might well be due to aestivation but more critical experiments would be required to elucidate this point.

VII. Selective action of fumigants on wireworms of different ages

In any experiments on the control of wireworms it is important to know whether a particular treatment has any selective action on the various instars. Are the young larvae killed more easily than those nearly fully grown or is there any particular stage of growth when the

Table XIII

High Field I experiment. Wireworms grouped according to length: before and after fumigation

(*a*) Difference in individual groups according to treatments.

	No fumigant ("*O*")				Chlorpicrin ("*C*")				("*S*")			
			Diff.				Diff.				Diff.	
Group	Before	After	Actual	%	Before	After	Actual	%	Before	After	Actual	%
1 (<0·5 cm.)	101	95	− 6	− 5·9	76	65	− 11	−14·4	137	20	−117	−85·3
2 (0·6–0·9 cm.)	351	441	+90	+25·7	330	234	− 96	−29·1	451	191	−260	−57·5
3 (1·0–1·3 cm.)	153	144	− 9	− 5·9	165	61	−104	−63·0	190	79	−111	−58·3
4 (1·4–1·6 cm.)	138	90	− 48	−34·8	116	74	− 42	−36·2	134	45	− 89	−66·5
5 (1·7–2 cm.)	56	23	−33	−58·9	53	29	− 24	−45·3	45	29	− 16	−35·5
6 (>2 cm.)	8	—	− 8	—	4	9	+ 5	—	8	2	− 6	—
Treatment totals, 40 soil samples 9 × 9 in.	807	793*			744	472*			965	366*		

(*b*) General composition (as % of totals)

	All treatments together		Separate treatments					
			"*O*"		"*C*"		"*S*"	
Group	Before	After	Before	After	Before	After	Before	After
1	13	11	12	12	10	14	14	6
2	48	53	44	56	45	49	46	52
3	19	17	19	18	22	13	20	21
4	13	13	17	11	15	16	14	12
5	6	5	7	3	7	6	5	8
6	1	1	1	0	1	2	1	1
	100	100	100	100	100	100	100	100

* These totals are slightly below those shown in Table IX as some wireworms were mutilated and could not be measured.

insects are more susceptible than at other times? Unfortunately it is impossible to differentiate rapidly between different species or to identify the particular instars, but failing any better method the length of the larva gives some indication of its age. As mentioned on p. 348, the wireworms were measured and divided into six arbitrary groups and Table XIII shows the distribution of the wireworms according to these groups on High Field I. It will be seen that the average composition of the catch in April after fumigation is much the same as in January before fumigation, with a slight increase in group 2 from 48 to 53 % and a reduction in group 3 from 19 to 17 %, but an examination of the grouping according to treatments shows that the fumigants do appear to have had some effect on the distribution. However, in assessing the differential effect of chlorpicrin and "*S*" on the various sizes of wireworms, due allowances must be made for normal growth, i.e. moving up from one group to another as shown for instance in group 2 in the unfumigated plots. "*S*" appears to have been most severe on group 1 which is reduced by 58 % whereas chlorpicrin only reduced it by 14 %. In group 2, "*S*" has effected a reduction of 58 % whereas chlorpicrin has reduced it by 29 % only. Group 3 appears to have been affected similarly by both fumigants. In group 4, the reduction by chlorpicrin (36 %) is no higher than that of the unfumigated plots (35 %), whereas "*S*" has reduced this group by 67 %. The numbers in the other two groups are too small for any legitimate comparison.

Table XIV shows the distribution of the wireworms over the various groups in the High Field II experiment. The actual numbers are much smaller than in High Field I, as the soil samples were smaller, fewer per plot and fewer replicates.

The sampling before fumigation was done in April and that after fumigation in June and as with High Field I the general composition of the counts was much the same in both.

Comparison of the first count in High Field II with the first count in High Field I shows, however, marked differences in some of the groups. Thus group 1 comprises only 8 % of the wireworms in High Field II whereas it reached 13 % in High Field I. Group 2 is 39 % compared with the 47 % of High Field I, but in group 3 the positions are reversed High Field II having 28 % compared with only 19 % in High Field I.

Taking groups 2 and 3 together there is no difference, these accounting for 66 and 67 % respectively, so that the differences in the individual groups might be merely the results of the normal growth between the months of January and April. In that case, however, it would be expected

Table XIV

High Field II experiment. Wireworms grouped according to length: before and after fumigation

(a) Difference in individual groups according to treatments

Group	No fumigant ("*O*") Before	After	Diff. Actual	Diff. %	Lime ("*A*") Before	After	Diff. Actual	Diff. %	Tar + lime ("*B*") Before	After	Diff. Actual	Diff. %	Am. carb. ("*C*") Before	After	Diff. Actual	Diff. %
1	7	8	+ 1	+ 14·2	13	15	+ 2	+ 15·4	9	6	− 3	− 33·3	9	11	+ 2	+ 22·2
2	41	47	+ 6	+ 14·6	37	41	+ 4	+ 10·8	50	22	− 28	− 56·0	46	25	− 21	− 45·5
3	48	39	− 9	− 18·7	17	29	+ 12	+ 70·5	36	24	− 12	− 33·3	31	30	− 1	− 3·2
4	21	16	− 5	− 23·8	18	19	+ 1	+ 5·6	17	24	+ 7	+ 41·2	13	11	− 2	− 15·4
5	20	13	− 7	− 35	11	15	+ 4	+ 36·4	11	11	0	—	12	13	+ 1	+ 8·33
6	0	0	—	—	2	0	− 2	—	2	1	− 1	—	2	2	0	—
atment tals, 16 soil mples	137	123	− 14		98	119	+ 21		125	88	− 37		113	92	− 21	

Group	Superphosphate ("*D*") Before	After	Diff. Actual	Diff. %	Naphthalene ("*E*") Before	After	Diff. Actual	Diff. %
1	6	10	+ 4	+ 66·6	12	4	− 8	− 66·6
2	32	46	+ 14	+ 43·7	73	23	− 50	− 68·5
3	30	20	− 10	− 33·3	39	11	− 28	− 17·5
4	17	18	+ 1	+ 5·9	14	7	− 7	− 50
5	12	10	− 2	− 16·7	9	15	+ 6	+ 66·6
6	—	—	—	—	1	2	+ 1	—
Treatment totals, 16 soil samples 6 × 6 in.	97	104	+ 7	—	148	62	− 86	—

Note. These treatment totals are below those shown in Table XII as some of the wireworms were dead or mutilated and could not be measured

(b) General composition (as % of total)

Group	All treatments together Before	After	Separate treatments "*O*" Before	After	"*A*" Before	After	"*B*" Before	After	"*C*" Before	After	"*D*" Before	After	"*E*" Before	After
1	8	9	5·2	6·5	13·3	12·6	7·2	6·8	8·0	11·9	6·2	9·6	8·1	6·5
2	39	35	29·9	38·3	37·8	34·5	40·0	25·1	40·7	27·2	33·1	44·3	49·3	37·1
3	28	26	35·5	31·7	17·3	24·4	28·8	27·2	27·4	32·7	30·9	19·2	26·3	17·7
4	14	16	15·3	13·0	18·4	15·9	13·6	27·2	11·5	11·9	17·5	17·3	9·5	11·3
5	10	13	14·6	10·5	11·2	12·6	8·8	12·5	10·6	14·1	12·3	9·6	6·1	24·2
6	1	1	0·0	0·0	2·0	0·0	1·6	1·2	1·8	2·2	0·0	0·0	0·7	3·2
	100	100	100	100	100	100	100	100	100	100	100	100	100	100

that the composition of the first count in High Field II would agree with the second count of High Field I on the unfumigated plots, both being taken in April, but as the figures in Table XIV B ("*O*", before) and in Table XIII B ("*O*", after) show no such agreement, it must be concluded that the differences are probably due to environmental factors.

Examining the distribution according to treatments, it will be noted that, on the untreated plots, there was an increase of the first two groups by about 14 % and a decrease in the other groups of from 18 to 35 %. No wireworms were found in group 6 either in April or June, but in June some of group 5 were pupating and in all groups individuals were moulting. Thus a moving up in the groups was actually taking place at the time of the second count and the pupating would produce a drop in groups 5 and 6. There was a general increase in all the groups on the limed plots, particularly in group 3. Tar and lime showed a good reduction in groups 1, 2, and 3 of which group 2 was the most (56 %), an increase in group 4 and no change in group 5. Ammonium carbonate gave a big reduction in group 2 (46 %) but no significant change otherwise. Superphosphate showed an increase in groups 1 and 2, a drop of 33 % in group 3 and no change in the other groups. Naphthalene appears to have reduced all groups equally drastically from 50 % in group 4 to 72 % in group 3.

VIII. Use of baits in estimating the density of the wireworm population

An important paper by Miles & Petherbridge was published in 1927 describing experiments with baits such as wheat, oats, potatoes and bran, as a means of assembling wireworms, attracting them from the roots of crop plants upon which they were feeding. The assembled insects were then killed by the application of calcium cyanide.

Miles & Petherbridge showed that there was considerable variability in the degree of attractiveness of various baits, potatoes being least attractive and wheat the most attractive. The preliminary investigation of an area of 4 acres, a typical Lincolnshire silt, made by taking 83 soil samples 6 in. square to a depth of 9 in., gave a total of 81 wireworms, about 170,000 per acre, the majority located within the top 3 in. of soil. The baits were set in rows 10 yd. long and 5 ft. apart, at a depth of 2–4 in., 21 rows in all. After 14 days the whole row was taken up and sifted for wireworms. In the total area 972 wireworms were assembled, representing 13,000 per acre, or 7·7 %. Further experiments showed that:

(*a*) Baits at 3–4 in. deep were more efficient than those at 2 in. (*b*) Bran and wheat attracted wireworms most speedily, while oats, peas, beans and potatoes increase in attractiveness from 6 to 12 days after setting. (*c*) From 60 to 80 % of the wireworms present in any area can be attracted to the baits, depending on the type of soil and crop grown. Calcium cyanide, applied at the rate of 2–3 lb. per 100 yd. of the bait row, killed 75–100 % of the wireworms assembled.

It would be a great saving of time and trouble if the density of the infestation on a set of plants could be ascertained by baiting instead of by direct counts on the soil. This would be possible only if the number of wireworms caught by the baits was a definite proportion of the population.

Experiment 1

The first experiment was made with a smaller concentration of bait than Miles & Petherbridge and using potatoes which they had found to be the least attractive, but convenient to handle. The experiment was done on the Knott Wood plots in July 1935. There were 25 plots altogether, each of $\frac{1}{60}$ acre. Crop, sugar-beet. Half-potatoes were used as baits. The baits were placed in between the rows of sugar-beet, about 3 in. from the surface, each plot being divided into three sections. Four baits at positions determined by random numbers were placed in each of three rows in each section, making twelve baits per section and thirty-six baits per plot. After 10 days the baits were lifted and examined, any wireworms being removed and any bad potatoes replaced by fresh ones. After a further period of 10 days the baits were lifted and examined once more. The experiment employed two men for 48 hr., being much less than the time occupied by actual counts on soil samples.

A comparison of the number of wireworms obtained by baiting with direct counts on the soil samples showed however that there was no simple relation between them, thus:

No. of wireworms (total of 5 plots)

Treatment	Direct counts (30 soil samples, 9 × 9 in.)	In baits: Actual	In baits: Per 100 direct count
Nil	79	56	71
Chlorpicrin	67	57	85
Chlordinitrobenzene	109	39	36
"*K*"	42	32	76
"*M*"	64	38	59
Mean	72·2	44·4	61·5

Looking at the last column, it is seen that for 100 wireworms found in the soil samples the number caught in the baits was not constant, although the ratios on the "*K*" plots and untreated plots are very similar, 76 and 71.

The experimental error was 40·1 % compared with 49·8 % by direct sampling. "*K*" gave the lowest number of wireworms both by the direct count and by baiting, and in both cases the reduction in numbers compared with the controls was nearly significant.

Experiment 2

High Field II, 48 plots of $\frac{1}{200}$ acre.

Here there was a much higher population and no crop to compete with the baits for the wireworms. In consequence more wireworms were obtained, although the number of baits per unit area was the same as in the first experiment.

Potato and cabbage-leaf baits were used, six of each kind per plot in two lines of three, both the locality of the lines and the positions of the bait in the lines being determined by randomization beforehand. The baits were put down on 22–24 May and examined on 27–31 May. Much rain fell between 22 and 27 May, so that the baits from blocks 1 and 5 that were examined on the day were very wet, probably causing fewer wireworms to be attracted. The baits were replaced and examined again on 5–7 June. The time occupied was about 62 hr. for a total of 576 baits, 9·29 baits per man per hr. This agrees very well with the time taken by the first experiment.

Table XV shows the number of wireworms caught in the different baits and compares these numbers with the population found by direct

Table XV

Comparison of bait catches with direct counts of numbers of wireworms

Total of 8 plots

Treatment	Direct counts (16 soil samples, 6 × 6 in.)	Baits: Actual			Baits: Per 100 direct		
		Pot.	Cab.	Total	Pot.	Cab.	Total
Nil	126	52	133	185	41	105	146
Lime	117	42	98	140	36	84	120
Tar + lime	92	79	116	195	86	126	212
Ammonium carbonate	90	37	97	134	41	108	149
Superphosphate	113	43	66	109	38	58	96
Naphthalene	65	48	110	158	74	169	243
Mean	100·5	50·2	103·3	153·5	50	103	153

counts on the soil. On the average the cabbage-leaf proved twice as attractive as the potato. From the analysis of co-variance there does not appear to be any real relationship between the number of wireworms obtained by the baits and the actual number in the soil. However, the highest proportion of the actual number of wireworms was obtained in the case of naphthalene, and tar + lime both with potatoes and cabbages. This indicates a repellent effect sending the wireworms into a more congenial environment, and considerably reducing any hopes that this baiting method could be used as a substitute for direct sampling in fumigation experiments unless a very large number of baits were used.

The object was not to trap the wireworms and destroy them as in Miles & Petherbridge's experiments, but it is interesting to ascertain whether the baits have captured an appreciable proportion of the wireworms infesting the plots. The total population calculated from direct soil samples is 225 wireworms per sq. yd. or 1,089,000 per acre. Of these, a total of 921 were obtained in the baits (potatoes and cabbages) from 48 plots of $\frac{1}{200}$ acre, equivalent to 3838 per acre, or 0·35 % of those found to be present in the soil by direct count, whereas Miles & Petherbridge obtained 7·7 % in their first experiment and from 60 to 80 % in subsequent trials. If the area of the paths is included in our experiments the proportion is considerably less than 0·35 %. Thus the baits have had no appreciable effect on the total number of wireworms present in the soil.

SUMMARY

1. This paper describes an attempt to find out whether it is possible to test chemical control measures against wireworms by a field technique similar to that used in fertilizer and varietal experimentation. A full account is given of three field experiments using old grassland on a heavy "clay-with-flints" soil .

The arrangements and sizes of the plots were as follows: (1) 5×5 Latin square, $\frac{1}{80}$ acre; (2) 3×10 randomized blocks, $\frac{1}{70}$ acre; and (3) 6×8 randomized blocks, $\frac{1}{200}$ acre.

2. Wireworm populations were ascertained in every case by sampling the soil before and after treatment. The Ladell flotation machine was found rather too small and a modification of the technique using oil drums was adopted. Later, a larger and improved form of the machine was used.

In the first experiment six samples ($9 \times 9 \times 5$ in.) were taken in each plot, making a total of 150 soil samples on each occasion. In the second,

four samples ($9 \times 9 \times 5$ in.) per plot were taken, making a total of 120 before treatment, but after treatment the size of the samples was reduced to $6 \times 6 \times 5$ in., the number of samples remaining as before. In the third experiment two samples ($6 \times 6 \times 6$ in.) per plot were taken, totalling 96 on each occasion.

Local control was introduced in the first two experiments. In addition preliminary experiments were carried out using potatoes and cabbage-leaf as baits as a means of estimating wireworm populations. No relationship was found between the number of wireworms obtained in the baits and the actual number in the soil.

3. The wireworms were grouped according to size in order to ascertain if there was any differential action of the fumigants on the various instars. No conclusive evidence was obtained.

The mean density of the original wireworm population was 65, 335 and 277 per sq. yd. respectively in the three experiments.

The uneven distribution of the wireworms in the soil resulted in high sampling errors accounting for most of the experimental error. This aspect of the work is discussed fully by W. G. Cochran in the Appendix. No relation was found between the *p*H of the soil and the density of the wireworm population.

4. The untreated controls showed changes of -23, $+1$ and -9 % between the first and second sampling respectively. The biggest drop was obtained when the second sampling was done in July and the smallest difference when the second sampling was done in May. It is suggested that the big drop in July might be due to a downward movement of the wireworms to escape the heat, but the evidence is insufficient to prove this point.

5. Fumigants "*K*" and "*S*" (mixture of *o*- and *p*-dichlorbenzene) reduced the population by 69 and 62 %, but had an adverse effect on the crop (sugar-beet) in both cases, due to insufficient interval between its application and the drilling of the seed. There was also a residual effect the following year, a reduction in the number of the wireworms and an increase in the plant stand (barley), although there was no significant increase in the final crop yield.

6. Chlorpicrin reduced the population by 46 and 33 %, but there was no harmful effect on the crops (sugar-beet and barley). No residual effect was detected.

7. Fumigant "*M*" (sodium cyanide) reduced the population by 45 % and increased the yield of the crop (sugar-beet). The residual effect was negligible.

8. Crude naphthalene ("creosote salts") reduced the population by 60 %, tar + lime by 29 % and ammonium carbonate by 20 %, on summer fallow.

9. Other treatments which all proved ineffective were chlordinitrobenzene, lime alone, and superphosphate.

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APPENDIX

THE INFORMATION SUPPLIED BY THE SAMPLING RESULTS

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1. *Introduction*

As the quotations at the beginning of Ladell's paper indicate, previous writers on the subject of wireworm control fully realized the need for estimating the wireworm population, but appeared to have no figures from which to assess the amount of work required to obtain a reasonably accurate estimate. An attempt to obtain such data was made by Jones (1937), who took samples with surface areas of 1, $\frac{1}{4}$ and $\frac{1}{16}$ sq. ft. respectively from a number of fields and compared the standard errors per sample. Jones finds, as one would expect, that the accuracy per sample increases as the size of the sample is increased; unfortunately, however, he does not balance this gain against the extra work required in taking larger samples, so as to find which size gives the best results per unit of work expended.

When a field experiment on the control of wireworms is under consideration, a preliminary sampling of the type which Ladell undertook is essential to determine the amount of work which is likely to be involved in estimating the effects of the treatments on the numbers of wireworms. The points on which a preliminary sampling may be expected to supply information are: (1) What size of treatment effect can we hope to detect with the amount of work done in the preliminary sampling? (2) If the treatment response which will be detected is considered too large, by how much must the sampling be increased to detect a treatment response of given size? If the standard of accuracy aimed at is found to involve too much labour, the postponement of the experiment must be seriously considered. (3) In a replicated field experiment of the type carried out by Ladell, the accuracy may be increased either by increasing the number of replications or by increasing the amount of sampling per plot. Which is the more profitable?

The purpose of this note is to show how the sampling technique used by Ladell enables us to answer these questions. We will consider in detail the results of the first sampling, which are given in Table I, p. 344.

When the experiment was started, it was of course unknown whether the distribution of wireworms was a random one over the whole experimental area. The experimental design used, a Latin square, was chosen

to take advantage of any regular gradients of infestation which might exist throughout the site, since differences in wireworm population between whole rows or columns do not affect the treatment comparisons. Further, the six soil samples taken per plot were restricted so that three fell in the north half and three in the south half of the plot; thus differences in infestation between these halves do not influence the treatment comparisons. This type of restriction, known as local control, is always worth while with new work, since one cannot lose anything in accuracy by it, and may gain substantially. The only limitation to its use is that at least four samples per plot are required to estimate the sampling error.

2. *The analysis of variance*

Before discussion, a complete analysis of variance is required. Owing to the small numbers of wireworms obtained per sample, their distribution is by no means normal, and before analysis the data ought to be transformed to some scale, such as square roots, on which they are approximately normally distributed. However, to keep the example as simple as possible, the analysis will be made on the numbers themselves; the conclusions are not altered thereby in this particular case.

The variation may be divided into: (I) Between-plot variation, which consists of variation between whole rows, with 4 degrees of freedom, variation between columns, with 4 degrees of freedom and the experimental error, with 16 degrees of freedom.* (II) The variation within plots between half-plots, derived from the differences of the totals of the north and south halves of a plot. As mentioned above, this variation does not enter into the experimental error, but it is worth calculating to see how much, if anything, has been gained by the local control. (III) The variation within half-plots, which constitutes the sampling error.

The first part, (I), is the ordinary analysis of variance of a Latin square and its calculation will not be given in detail here, as it is described with full numerical working in many text-books, such as that by Fisher & Wishart (1930). The ordinary "treatments" and "error" terms should be combined, as there are no treatments. If, however, the first sampling contained different treatments, the error term alone would be used. This analysis will be on a single plot basis (total of six samples).

To obtain (II), first take the differences (ignoring sign) between totals of the south and north halves of each plot. These are shown in Table I in the columns headed (S – N). The sum of the squares of these twenty-

* Plots 103–107 form the first row, and plots 103, 108, 113, 118 and 123 form the first column, etc.

five differences is 2269 and is on a single plot basis. The sampling error (III) may be obtained by a subtraction. Calculate the sum of the squares of all 150 samples; this comes to 3767 and is on a single *sample* basis. Multiplying by 6, to bring this to a single *plot* basis, gives 22,602. Subtract the product of the grand total, 607, and the general mean 24·28. This gives the total sum of squares 7864·04 with 149 degrees of freedom. The sampling variation may now be obtained by subtracting (I) and (II) from the total and has 100 degrees of freedom.

The complete analysis of variance is as follows:

	D.F.	Sums of squares	Mean square	S.E.
Rows	4	515·44	128·86	—
Columns	4	523·44	130 86	—
Experimental error	16	712·16	44·51	6·672
Between half-plots	25	2269·00	90·76	—
Sampling error	100	3844 00	38·44	6·120
Total	149	7864·04		

3. *The information supplied by the preliminary sampling*

The first point to notice is that the experimental design considerably improved the accuracy of the results, since the mean squares due to rows, columns and differences between half-plots are all substantially above the experimental and sampling mean squares. Had the experiment been randomized completely within the site chosen, on the ground that the wireworm distribution was a random one, the experimental error (with local control) would have been

$$\tfrac{1}{24}(515{\cdot}44+523{\cdot}44+712{\cdot}16)=72{\cdot}96 \text{ instead of } 44{\cdot}51.$$

Further, if no local control had been used, the sampling error would have been

$$\tfrac{1}{125}(2269+3844)=48{\cdot}90 \text{ instead of } 38{\cdot}44.$$

Thus an estimate of the experimental error with complete randomization and no local control is

$$72{\cdot}96+49{\cdot}90-38{\cdot}44=83{\cdot}42 \text{ instead of } 44{\cdot}51,$$

so that the accuracy of the experiment has been nearly doubled by the design.

The experimental error is 6·672 per plot and the standard error of a treatment mean (5 replicates) is $6{\cdot}672/\sqrt{5}=2{\cdot}98$, which is 12·3 % of the general mean (24·28). Thus the standard error of the difference between two treatments is 17·4 % of the general mean. To find the percentage difference which would be detected at the 5 % level of significance, this must be multiplied by 2·120, the value of "t" for 16 degrees of freedom.

Thus an apparent difference of 37 % between two treatment means will be significant.

When we are aiming at a given standard of accuracy, a further point, slightly more subtle, must be appreciated. If the *true* difference between say one fumigant and the control were 37 %, then in a number of experiments the estimated difference would vary about this value, being above it in half the experiments and below it in half. Thus in an individual experiment, a *true* difference of 37 % has only a chance of one in two of being detected as significant. The question arises, how large must the true difference be so that it will almost certainly be detected, say in nineteen experiments out of twenty? The difference must clearly be so large that only in 5 % of cases will the observed value of t fall below 2·120.

If x is the observed treatment difference, s its estimated standard error and μ the real treatment difference, then the tabulated $t=(x-\mu)/s$ and we want the value of t which is exceeded in all but 5 % of cases. From the t-table for 16 degrees of freedom we see that the value of t lies inside the limits $\pm 1{\cdot}746$ in all but 10 % of cases. Since the t-distribution is symmetrical, the value must therefore exceed $-1{\cdot}746$ in all but 5 % of cases. Thus the real difference must be such that when $(x-\mu)/s=-1{\cdot}746$, then x/s, the observed t, is 2·120. This gives $\mu=3{\cdot}866\,s=16{\cdot}29$, which is 67 % of the general mean.

Thus if we wish to be reasonably certain of detecting a response to a treatment, the response must be at least 67 %. This answers the first question in the introduction.

To consider the second question, suppose that we wished to detect a difference of 50 % in 95 % of cases. The standard error per plot would have to be reduced to $\frac{50}{67}$ of its present value and the experimental variance reduced in the ratio $(\frac{50}{67})^2$, i.e. to 24·8.

The third question concerns the best way to do this. The sampling variance, 38·44, accounts for all but 6·07 of the experimental variance. Thus doubling the sampling per plot, but keeping the size of the experiment fixed, would reduce the experimental error by $\frac{1}{2}$ (38·44) = 19·22, i.e. to 25·3. On the other hand, keeping the *total* amount of sampling fixed, but doubling the size of the experiment, would reduce the experimental error by only $\frac{1}{2}$ (6·07) = 3·04, i.e. to 41·47. There is thus a much smaller return from doubling the experimental area (using say two 5 × 5 Latin squares) than from doubling the amount of sampling. This must, however, be balanced by the experimenter against the expenditure of time and labour in doubling the sampling and the experimental work. It is

probable that with this type of work the former will be the more exacting operation and may even be a limiting factor to the size of the experiment.

In any investigation in which the major portion of the work is taken up in handling samples, the best return for the labour expended is obtained when the sampling variance accounts for all, or almost all, the experimental variance.

The only change in Ladell's technique suggested as a result of the preliminary sampling was to increase the local control at the next sampling by taking two samples per third of a plot instead of three per half-plot. The precision attained on the treatment comparisons was considered sufficient, as only large treatment effects would be of commercial interest, and in any case the experiment was regarded as one mainly on technique. As the experimental error was almost entirely due to variation between samples, no reduction in the amount of sampling could be recommended.

4. *The experimental and sampling errors*

The results of Ladell's experiments from the point of view of sampling technique are summarized in Table II. In most cases a high proportion of the experimental error was due to errors in sampling. As the amount of sampling was the limiting factor in these experiments, this result is gratifying and means that the labour of sampling could not have been decreased without a considerable sacrifice in accuracy. The experimental errors are high; nevertheless certain significant treatment effects were detected.

The amount of sampling varied from $\frac{1}{4}$ to $\frac{1}{2}$% and the areas within which the sampling variation was taken ranged from $\frac{1}{200}$ to $\frac{1}{120}$ acre.

For comparison with other workers' data the sampling errors are best expressed in terms of a single sample, as shown in the table below.

Sampling errors per sample

Size of sample in.	Size of area sampled acres	W Number of wire-worms per sample	E Sampling error % per sample	$E\sqrt{W}$
9 × 9	$\frac{1}{120}$	4·0	61	122
9 × 9	$\frac{1}{180}$	2·4	83	129
6 × 6	$\frac{1}{120}$	1·2	96	105
9 × 9	$\frac{1}{140}$	21·0	38	174
6 × 6	$\frac{1}{140}$	6·2	48	119
6 × 6	$\frac{1}{200}$	7·7	74	203
6 × 6	$\frac{1}{200}$	6·3	72	181

When an experiment of this type is contemplated with new material of unknown variability, it is sometimes useful to note that a lower limit

to the sampling error may be obtained from theoretical considerations alone. If the wireworms were scattered at random throughout the sub-plots sampled, the numbers found per sample should be distributed in a Poisson series distribution. For this distribution the mean W is equal to the variance, so that the standard error E % per sample should be $100/\sqrt{W}$. Other sources of variation may, of course, increase this error.

The values of $E\sqrt{W}$ for Ladell's experiments are given in the last column of the table. They show quite a close agreement with theory in the first experiment, where the numbers per sample were very small, but with higher numbers per sample other sources of variation become important.

Jones's figures agree remarkably well with these, despite the fact that he was sampling from much larger areas. For the 25 sets of 100 sq. ft. samples in his Table III, the means ranged from 0·22 to 10·78 per sample and the values of $E\sqrt{W}$ from 85 to 237. The values of $E\sqrt{W}$ also increase as the means increase; in fact, apart from one or two widely aberrant sets, a good summary of Jones's results may be obtained by taking a linear regression of the form

$$E\sqrt{W}=a+b\sqrt{W},$$

where a and b are constants.

This agreement between results obtained under widely differing conditions gives one confidence in recommending Jones's and Ladell's sampling errors to workers who are planning field experiments on wireworms and wish to obtain a preliminary idea of the scale on which they will have to sample. This scale should, of course, be reconsidered as soon as the results of their first sampling are available, by the method indicated in section 2. It should be noted, however, that Jones's sampling technique, which involved a systematic rather than a random distribution of samples, is not recommended for sampling work in field experiments.

Summary

In any field experiment which involves sampling of a laborious nature, it is important to know as soon as possible what degree of accuracy in the treatment mean values will be reached with a given amount of work, how much work must be done to reach a given standard of accuracy and how best to distribute one's resources between the amount of sampling and the amount of replication.

The first sampling, whether it contains experimental treatments or is uniformly treated, can supply information on all these points if

properly carried out. Ladell's first wireworm sampling is taken as a simple numerical example of the way in which these questions can be answered with the help of an analysis of variance.

The sampling and experimental errors of Ladell's experiments are discussed. The sampling error accounts for a large proportion of the experimental error in most cases, as it is always advisable where the labour involved in sampling is high.

Ladell's sampling errors agree well with those obtained under widely different conditions by Jones, and both may be recommended to other workers as an indication of the amount of variability to be expected in field sampling for wireworms.

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INVESTIGATIONS INTO THE NUTRITION OF THE ASH-BARK BEETLE, *HYLESINUS FRAXINI* PANZ.

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Introduction

No work has been done so far on the physiology of the nutrition of the bark-feeding Coleoptera. This is not surprising, considering that it is only very recently that attention has been given to the nutrition of some of the wood-feeding insects proper, which were described by Uvarov (1928) as "insects of entirely unknown feeding habit". Since then, papers by various workers have dealt with investigations into the nutrition of some of the wood-borers, but the nutrition of the bark beetles (Scolytidae) has never been studied despite its great interest, and the suggestion by Escherich (1923) and Munro (1926) that bark-beetle attack is only a secondary symptom confined to trees already in a pathological condition and is very rarely found in sound trees. It is a well-known fact, used in the control of the pest, that the insects go to felled trees if available rather than to living ones (Escherich). If it were possible to get a clear knowledge of the substances serving as their food, of the attraction of the insect by the plant, and the physiological relationship between them, new aspects of the ecology of both plant and insect would be opened, which might lead to better methods of control.

Accounts of the life history and anatomy of *Hylesinus* (*Leperisinus*) *fraxini* can be found in the writings of Escherich (1923), Munro (1926) and others. There are two distinctly different types of nutrition:

(1) "Brutfrass" which is the nutrition of the larva, and with which is linked up the "Ernährungsfrass" of the adult.

(2) "Reifungsfrass" which serves the young imago to reach sexual maturity.

Only the first type of nutrition is dealt with in this paper. The carbohydrate metabolism by frass analysis was studied quantitatively only in the larvae. However, the similarity of the enzymes and the

qualitative results of the analysis lead to the conclusion that there is no fundamental difference between larval and adult metabolism.

The nutrition of the ash-bark beetle, *Hylesinus fraxini*, has been studied in two ways:

(1) By an investigation into the enzymes of the alimentary canal.

(2) By a comparative analysis of the bark and of the frass of the insects, the word "frass" here meaning the total contents of the food tunnels.

SOURCES OF ERROR

These may be considerable in this kind of work, and have to be taken into account when assessing the value of the results. The following have to be considered:

Inaccuracy of methods. The biochemical methods employed are not very well worked out, and are usually very specific; the presence of other substances may affect results to a large extent, and the variation between duplicates or triplicates of the same estimation is usually considerable.

Variation in material. Though care was taken to eliminate this factor as much as possible by taking frass and bark always from the same pieces, and by making as many estimations as possible, it must be remembered that the chemical constitution of plants varies according to age, position, etc., to an unknown degree.

Metabolism of the material. As seasoning of the wood proceeds chemical changes occur in it, such as the breakdown of certain reserve carbohydrates. It is often difficult to decide how much of this breakdown is due to the metabolism of the bark and how much to the digestion by the insect.

Bacterial action. The frass in its partly broken-down form is much more susceptible to bacterial attack than the original bark. It is impossible to say to what amount changes have occurred in it after it has left the insect gut.

Composition of food-tunnels. These in places pass into the sapwood. Although there is probably not much sapwood passing through the gut, all results may be somewhat too high in their difference from the original bark, as the cellulose content, which has been made the basis of the calculation (see later), is higher in sapwood than in bark. No material was taken from the distal end of the food-tunnels, where the larva bores right into the sapwood to pupate.

Investigation of the enzymes of the alimentary canal

For these experiments a tissue suspension was prepared in the following way. For each test the guts of about twenty larvae or adults were dissected out and ground up with a few drops of distilled water in a solid watch-glass by means of a glass rod to give about 0·5 c.c. The suspension had $p\text{H}=7{\cdot}6$, which was found to remain unchanged in all tests after addition of the substrate without buffering, except in the peptase and hemicellulase tests. The suspension was divided into equal parts in two small glass tubes, one of which was heated for a few minutes in a boiling-water bath to kill the enzyme, for control. Equal amounts of the substrate were then added to each of the tubes together with a drop of toluene to prevent bacterial action, and both tubes were left in an incubator at 37° C. for 48 hr., after which time they were tested.

The following tests were carried out:

Peptase.

(*a*) A few particles of carmin-fibrin were added to the tissue suspension. If digestion took place the carmin would be liberated into the solution. The solution was kept acid with 2 drops of $N/10$ acetic acid (Cole, 1933, Ex. 262).

(*b*) An equal amount of acid casein solution, prepared according to Cole (1933, Ex. 261), was added to the suspension, and the test and its control were titrated with 10% Na-acetate until a precipitate was obtained. If digestion took place a precipitate would be obtained later in the test than in the control, as casein would have disappeared from the former.

Tryptase.

(*a*) A few particles of Congo-red fibrin was added. Coloration test as under Peptase (*a*) (Plimmer, 1915, p. 474).

(*b*) Casein test: a neutral casein solution was added in equal parts to the suspension. Precipitation test with 1% acetic acid (Cole, 1933, Ex. 273).

Peptidase.

Equal amounts of 1% peptone solution were used as substrate. The disappearance of peptone from the digest was tested with the Biuret test (Cole, 1933, Ex. 277).

Lipase.

An emulsion of olive oil in water was used as substrate. The appearance of fatty acids through the action of lipase was tested by decoloration

of phenolphthalein and, if necessary, titration with $N/50$ NaOH (Plimmer, p. 475; Cole, Ex. 184).

Saccharase.

1 % cane-sugar solution was used as substrate. Tested by reduction of Benedict's solution.

Maltase.

1 % maltose solution was used as substrate. The osazone test (Plimmer, p. 265; Cole, Exp. 139) was carried out with the digest. If cane sugar was broken down, crystals of glucosazone would appear in the test.

Lactase.

1 % lactose solution was used as substrate. Osazone test as above.

Dextrinase.

1 % dextrin solution was used as substrate. Osazone test as above.

Amylase.

1 % starch solution was used as substrate. The disappearance of starch was tested with iodine solution.

Amylo-hemicellulase.

This test was carried out to test the behaviour of the gastric juices to α-glucans of higher molecular weight than starch. A few particles of amylo-hemicellulose (see Clayson & Schryver, 1923) were added to the suspension, and digestion was tested with iodine solution as in the case of starch, the substrate giving a bright blue coloration in this test.

Hemicellulase.

Hemicellulose "B" from ash bark, prepared in the course of the chemical analysis, was used as substrate. The suspension was buffered to $p\mathrm{H} = 7{\cdot}6$ with acetate buffer. Digestion was tested for with Tollens's test and Benedict's test.

Cellulase.

(*a*) A suspension of filter-paper was used as substrate and the test made with chlor-zinc-iodine solution (Schulz solution).

(*b*) Sections through the stem of a green plant were exposed to the actions of the enzymes of the suspension, and later stained with Schulz solution, and examined under the microscope. Failure of the cell walls to stain purple indicated that the cellulose in them had broken down.

(*c*) A few milligrams of finely divided cellulose pulp ("poudre" Prat, Courze) were weighed into a known quantity of suspension and, after

digestion, any reducing sugars present were estimated by reduction following Bertrand's method.

The results of all these tests are expressed in Table I.

Table I

Determination of enzymes of Hylesinus fraxini

Enzyme	Result	Enzyme	Result
Peptase	Absent	Lactase	Very weak
Tryptase	Present	Dextrinase	Present
Peptidase	Present	Amylase	Weak
Lipase	Weak	Amylo-hemicellulase	Very weak
Saccharase	Present	Hemicellulase	Present
Maltase	Present	Cellulase	Absent

After the chemical analysis of the bark had shown that starch had disappeared from the bark before it is attacked by *H. fraxini*, it seemed advisable to measure the quantitative strength of the group of enzymes attacking α-glucans, to investigate whether there was a connexion between the delay in attack (i.e. until the disappearance of the starch) and the strength of the enzymes. At the same time it was thought desirable to compare the strength of the hemicellulase with that of the lactase, as galactan forms the greater part of the hemicellulose. In view of the fact that some authors, such as Bernhauer (1933), show galactan with the β-linkage, it was thought possible that the enzyme in question would be most nearly related to that attacking lactose, i.e. a β-galactoside. For these experiments the guts were treated as before, and an equal amount of the suspension measured into each tube with a micropipette. The substrate was weighed in. After 48 hr., all reducing substances were measured by the Bertrand method. The difference in reduction between test and control gave the action of the enzyme.

The result is expressed in Table II.

Table II

Quantitative action of several carbohydrases

Material	Weighed in g.	Reduction after 48 hr. in glucose g.	Net reduction due to enzyme %
Dextrin	0·032	0·030	90
Control	0·035	0·014	—
Starch	0·032	0·018	56
Control	0·033	0·000	—
Lactose	0·029	0·019	27
Control	0·033	0·017	—
Hemicellulose "B"	0·030	0·009	30
Control	0·038	0·000	—

COMPARATIVE ANALYSIS OF BARK AND FRASS

A. *Carbohydrates*

The utilization of carbohydrates by wood-feeding insects has been studied by the method of comparative analysis by Campbell (1929) working on *Lyctus* spp. and *Xestobium rufovillosum*, and by Norman (1936) working on *Xestobium*. Part analysis has been carried out by Ripper (1930) working on some cerambycid larvae and *Xestobium*, by Falck (1930*a*, *b*) in work on the cellulose digestion of *Hypotrupes bajulus* and of *Anobium*, and by Mansour & Mansour-Bek (1934*a*, *b*) in their study of the nutrition of *Macrotoma palmata* and *Xystrocera globosa*.

For the present investigation, ash bark was ground up to pass a 60-mesh sieve but to be retained by a 90-mesh sieve. The frass of the larva passes 60 mesh easily, but is partly retained by 90 mesh. The greater part of the adult frass is retained by a 60-mesh sieve. General appearance of the particles and the general analysis suggests that only part of the adult frass has passed through the gut. It is not definitely known how much of the larval frass actually passes through the gut, but it may be assumed that the greater part does so.

The methods employed are given in the text-book by Hawley & Wise (1926) on the chemistry of wood, and the structure and properties of the carbohydrates concerned and their biochemical metabolism has been described by Bernhauer (1933) and others. An account of the chemistry of the major carbohydrate constituents of ash bark and the method for their estimation has already been given elsewhere (Buston & Hopf, 1938) and only the general methods are here briefly outlined.

The following fractions of the material were distinguished:

(1) Hot-water-soluble material, containing all the simple sugars, together with dextrins, glucosides and wood starch (cf. Campbell, 1935).

(2) Pectic substances, obtained by extraction with 0·5 % ammonium oxalate.

(3) Alkali-soluble substances, mainly hemicellulose.

(4) Cellulose (including inseparable pentosans).

(5) Lignin.

The material was first extracted with water under a reflux condenser at about 90° C. four to six times for 5 hr each. The extraction with ammonium oxalate was made at the same temperature for 6 hr. The remaining substance was extracted with *N*/1 NaOH several times (until the extract was colourless), 5 hr. each.

The water-soluble group was further subdivided by adding nine parts of alcohol to one part of the extract, which throws down all the higher polysaccharides. After filtering off, reduction estimations were made with the filtrate before and after hydrolysis with weak sulphuric acid, giving the amounts of simple sugars (monosaccharides) and of the more complicated (disaccharides, simple oligoses) respectively.

The alkali-soluble fraction was first acidified to litmus with acetic acid, the precipitate of hemicellulose "A" thus obtained filtered off, and five parts of alcohol were added to the filtrate. A precipitate of hemicellulose "B" was obtained. This was filtered off, and the same amount of alcohol was added again, giving a precipitate of hemicellulose "C". The separation of "B" and "C" can naturally not be regarded as quantitative. In each hemicellulose the amount of uronic anhydride was measured by determining the carboxyl groups, and the pentosan constituents by the furfural method of Tollens, as modified by Norris & Resch (1935). (See also Preece, 1931; Angell *et al.* 1936.) The furfural due to urone was allowed for in this estimation. The rest was regarded as consisting of hexosans. Lignin was estimated by Willstätter's method; cellulose by chlorination according to Cross and Bevan's method.

The same methods of fractionation and analysis were applied to the frass and the bark.

To determine the actual loss in weight of the frass by passing through the insect gut, it was assumed that cellulose, which was found to be unattackable by the gastric enzymes, remained constant. As the cellulose-lignin ratio seemed to remain constant within the limits of error this assumption seemed justified.

There was some variation in the different specimens of bark investigated, according to the age of the attacked specimen, its moisture content while exposed after felling, and the progress of the seasoning metabolism. The summary of a typical example, which represents a fair average of the determinations made, is given in Table III.

The simple sugar fraction seems to consist mainly of glucose. It is difficult to say anything definite about the nature of the alcohol-soluble, and even less about the alcohol-insoluble, polysaccharides. However, two facts seem to be important in this connexion. First, the bark in the state in which it is ready for insect attack contains no starch. The iodine test was always negative. Fresh ash bark always shows a clear reaction. The disappearance of starch in felled timber by seasoning has been recorded before by Mer (1903), Wilson (1930) and Henderson (1935). Second, the alcohol-insoluble polysaccharides of the unattacked bark in fresh trees

contain little or no pentose constituents. With the seasoning of the wood, pentose appears in increasing quantities in this fraction, probably derived from the breakdown of reserve material, chiefly hemicelluloses. However, the frass always contains more pentose than the bark and shows a positive reaction to the orcinol test at a time when this fraction from the unattacked bark seems to be still practically free from pentose. It is most likely that the fraction consists mostly of α-glucans of the dextrin type, though this could not be proved either by acid-hydrolysis or by the takadiastase method described by Denny (1934) which Campbell (1935) employed successfully.

Table III

Analysis of bark and frass (general)

	Material		
	Bark %	Frass %	Frass in % of original bark* %
Water-soluble fraction:			
Simple sugars	5·32	4·85	4·00
Alcohol-soluble polysaccharides	4·86	1·65	1·36
Alcohol-insoluble polysaccharides	8·05	6·2	5·12
Other substances	11·52	13·6	11·2
Total	29·75	26·3	21·7
Alkali (4% NaOH)-soluble fraction:			
Hemicellulose "A"	2·65	3·55	2·93
Hemicellulose "B"	13·6	10·65	8·78
Hemicellulose "C"	3·12	1·8	1·49
Total hemicellulose	19·37	16·0	13·2
Pectin (as Ca-pectate)	3·24	4·6	>3·8
Lignin	16·5	21·5	17·5
Cellulose	32·5	39·4	32·5
Total ash	6·42	7·3	6·01
Total pentosans	10·6	12·2	10·08
Protein†	2·88	1·43	1·18

* Based on the assumption that cellulose remains constant, in which case frass = 82·5% of original bark.

† See Table V.

The group comprised in Table III under the heading "other substances" in the water-soluble fraction probably consists of material other than carbohydrates together with those carbohydrates which have resisted 12 hr. hydrolysis. No details whatsoever (except for the proteins) about its nature are available.

The pectic substances consist for the greater part (over 80%) of pure polyuronides. The values given are calculated as calcium pectate and are therefore not true percentages of the material.

The hemicelluloses of the ash bark are built up of three main constituents: galactan and mannan (hexosan), araban, and uronic acid. They differ mostly according to the distribution of these groups and by their molecular size.

The utilization of the hemicellulose constituents will be seen from the figures in Table IV, where the percentages found in bark and in frass are given. The percentages of the unattacked bark have again been calculated under the assumption that:

$$\frac{\text{frass}}{\text{bark}}=\frac{\text{cellulose of bark}}{\text{cellulose of frass}}=\frac{32{\cdot}5}{39{\cdot}4}.$$

Table IV

Analysis of bark and frass (Hemicelluloses)

Hemi-cellulose	Constituent	Bark: % of respective hemi-cellulose	Bark: % of unattacked bark	Frass: % of respective hemi-cellulose	Frass: % of unattacked bark
"A"	Hexosan	—	—	—	—
	Uronic anhydride	—	—	—	—
	Pentosan	95	2·6	—	—
	Total	100	2·65	100	2·93
"B"	Hexosan	65	8·77	40	3·5
	Uronic anhydride	17	2·37	29·9	2·63
	Pentosan	18	2·46	30·1	2·64
	Total	100	13·6	100	8·78
"C"	Hexosan	65	2·03	—	—
	Uronic anhydride	20	0·62	—	—
	Pentosan	15	0·47	—	—
	Total	100	3·12	100	1·49

From the values giving the constituents in percentages of the dry weight of unattacked bark it will be seen that as the variations in the amounts of urones and pentosans are within the limits of error necessarily connected with work of that kind, the decrease in hexosans is very considerable and must be regarded as due to utilization of hexosan by *Hylesinus fraxini.*

The frass numbers have only been worked out for "B", as this is present in larger quantities and must necessarily play a greater role in nutrition than "C". However, the results should be the same in principle in both cases.

The cellulose was regarded as one unit and no distinction was made between the β-glucan proper and the pentosan groups linked up with them. From what is known about them and the results of the enzyme tests and about the pentoses of the alkali-soluble group, it seems very

unlikely that these molecular chains are attackable by the insects in question and that therefore further division is of no interest in this investigation.

The "total pentosan" value was inserted following Campbell (1929). It might better be described as the total yield of furfuralphloroglucide, were it all due to pentosans. In fact, some of it will be due to uronic anhydride, the amount of which it is impossible to determine because of the interference of organic acids etc.

B. *Proteins*

The results of this investigation have already been referred to in a letter to *Nature* (Hopf, 1937) dealing with the protein digestion of wood-boring insects.

The total nitrogen was first determined by the micro-Kjeldahl method. The material was then extracted for several hours with hot water to which some phenol was added and trichloracetic acid to throw down the protein. From the filtrate the non-protein was determined by the micro-Kjeldahl method, the difference being regarded as protein nitrogen. The protein number was obtained by multiplying this value by 6·25.

Table V

Analysis of bark and frass (Proteins)

Sample	Total N %	Non-protein N %	Protein N %	Protein %	% of unattacked bark
Ash bark	0·54	0·08	0·46	2·88	2·88
Frass of larva	0·41	0·182	0·288	1·43	1·18
Frass of adult	0·68	0·326	0·354	2·21	—

A considerable amount of ammonia was found in the excretions, which may have been derived from uric acid by bacterial action. In no case was there enough uric acid to account for the total excretory nitrogen. Urea was never present.

No work has been done on fat and similar substances in the bark, but they do occur there.

DISCUSSION

The sources of error mentioned above may certainly affect the quantitative details of the results, but it is hardly probable that they make a principal difference, especially as the two methods of investigation, i.e. the enzymological and the analytical, yield results pointing in the same direction.

Apparently *Hylesinus fraxini* is able to make use of a very varied supply of carbohydrates. There is a weak α-glucanase which, though it is strong enough to break down starch, is by no means as strong as that found in many other insects, where the iodine test in the digest is often negative after a few minutes. It is also strong enough to digest quantitatively and in a short time the carbohydrates similar to starch but of shorter chain-length, which are probably present in the bark in the form of dextrins and other oligoses. A considerable decrease is found in this fraction of the frass. The actual utilization of this group is certainly much greater than is indicated in the decrease in the alcohol-insoluble sugars, but this fraction is filled up again with breakdown products of the higher groups, i.e. the hemicelluloses, as the increased presence of pentoses in it in the frass indicates. As there is no starch present in the bark attacked, the trees in this state seem to be already in a pathological condition, which gives support to Munro's suggestion that the insect only attacks such trees as a secondary pest. Fresh ash bark always shows a positive reaction to the iodine test. It might appear, therefore, that the beetles are attracted to trees offering them, in the form of already partly broken-down starch, a food more easily digestible. This is underlined by the observation that α-glucosides of chain-length even greater than starch (amylohemicellulose) are still less attacked by the enzymes. The question remains open whether the starch breakdown as such, through fermentation or otherwise, supplies the attractant to the beetle.

It is interesting to contrast this behaviour with what is known about the feeding habits of some of the wood-boring insects proper. *Lyctus*, for instance, seems to feed mostly on starch. The plant-physiological work by Mer (1903), Wilson (1930), and Henderson (1935), the analysis by Campbell (1929), and especially the feeding and extraction experiments by Parkin (1936), have proved that *Lyctus* depends on an ample supply of starch and will not attack wood which is free from it.[1] Many other wood-boring insects also seem to depend on starch (*vide* Mansour & Mansour-Bek, 1934). In the case of the ash-bark beetle, the mode of nutrition is entirely different and it shows a decided preference for already seasoned wood, and will even attack an old specimen twice, chewing frass of the previous broods again, rather than attack fresh bark nearby.

[1] In this connection, it is interesting to draw attention to the statement by Munro (1928) that with the exception of *Hylotrupes bajulus* all the longicorn beetles commonly introduced into this country prefer unseasoned timber as a breeding ground, and for that reason they rarely increase in the timber yards, and the injuries they cause do not extend to other timber in the vicinity. *Hylotrupes* has been proved by Falck (1930*b*, see later) to utilize cellulose.

That there is a considerable loss of alcohol-soluble sugars of the disaccharide and similar types in the frass is quite natural. There are probably very powerful enzymes for these groups present, which split up all breakdown products from higher groups. The simple sugars do not decrease in the same proportion, showing that the enzymes split up more than the body is able to assimilate and that, therefore, a part of the digest is allowed to leave the gut with the frass. There is, of course, always the possibility that the presence of reducing substances other than sugars increases the values in this fraction, especially in the frass.

The utilization of the hemicelluloses is especially interesting as, according to a widely accepted theory, these bodies have arisen by a biochemical oxidation, which first transformed the alcohol groups of some links of a hexose chain into carboxyl groups yielding uronides, and then by further oxidation split off carbon dioxide to give a pentose link. As can be seen from Table IV, only the hexose (galactose) links are utilized. Their disappearance partly breaks the chain, so that pentoses appear together with carbohydrates of as small molecular weight as the water-soluble, alcohol-insoluble saccharides. The apparent increase in hemicellulose "A" can probably be explained by "B" and "C" break down products, containing much pentose and little urone, having thus acquired the approximate solubility of the "A" group which is less than that of "B" and "C."

Hemicellulases are well known in insects. Campbell (1929) and Norman (1936) found that *Xestobium* utilizes hemicellulose, but the only case where a hemicellulase has been more clearly defined in a wood-boring insect is that of the lichenase which Ullmann (1932) found in *Cossus cossus*—as lichenin may be regarded as related to the hemi-celluloses.

The process in the *Hylesinus* gut is probably this, that the enzymes split off the hexose units of the chain. The rest of this chain goes either into fractions small enough to appear in the water-soluble group, or in the hemicelluloses of smaller molecular weight, possibly in "A", or stays in "B" with a decrease of the hexose constituent. It seems quite feasible that part-oxidation during the process converts the urone constituents of the chain partly into pentose units, with corresponding decrease of solubility. Hence the increase in hemicellulose "A". "C" is relatively more strongly attacked than "B" as it is of smaller molecular size, but, as it is present only in small quantities, it does not play the same role as "B" in the nutrition.

The presence of only a weak lactase can be reconciled quite well with the utilization of the hemicelluloses. The two enzymes are probably quite different, especially as it is only assumed that we have to deal with

a β-galactanase. The breakdown may go directly to free galactose without passing through the units which necessitate a β-galactosidase.

Cellulose and carbohydrates of similar type are certainly not attacked. Enzymes attacking these complexes have never been recorded in insects of this type. Cellulose digestion is common in wood-boring insects and has been recorded in certain cerambycids (Ripper, 1930; Falck, 1930*b*; Mansour & Mansour-Bek, 1934*a*, *b*; Horn, 1932) and anobiids (Ripper, 1930; Falck, 1930*a*; Campbell, 1929; and Norman, 1936) by means of chemical analysis. It is not quite certain whether the digestion is due to enzymes secreted by the insects or by bacteria or other micro-organisms with which they live symbiotically. Cellulases seem to be present in certain cerambycids, but their presence in some other cases, in which they have been recorded, cannot yet be regarded as actually proved. I was not able to demonstrate the presence of the enzyme in locusts (Biedermann, 1919) or in the larva of *Anobium*, where Falck (1930*a*) records a disappearance of cellulose in the frass by analysis, while the method gave very clear results in the case of *Helix pomata* (Karrer, 1925).[1]

The utilization of the proteins from the bark seems to be in accordance with the strength of the enzymes and the manner in which the carbohydrates are attacked. It is a surprising fact that the adult beetle actually excretes more nitrogen than it takes up, but it must be remembered that the adult frass examined was obtained from beetles within 2–3 weeks after emergence from the pupa. It seems that the insect gets rid of the waste products of metamorphosis in a way resembling the deposition of the meconium in Lepidoptera. Possibly analysis of the "Reifungsfrass" of the young adults before mating and oviposition, would give quite different results, but as it was not possible to breed *Hylesinus* in captivity, insufficient material for this investigation was available. The whole question of the nitrogen supply of insects is still in need of further research. A definite discrimination between the different proteins available is required, and the problem of excretion, in particular, seems to offer a wide scope for further research. No final conclusions can be reached before this question is cleared up.

It is also necessary in the study of the ecological aspect of the question to establish definitely the connexion and interaction of the physiology of the plant and of the insect with regard to the nutrition of each. There may naturally be many other factors acting as attractants outside the starch breakdown, or even outside the food supply. An accurate and

[1] Since writing this paper Schlottke (1937) states that no cellulase occurs in *Saltatoria* investigated by him.

detailed investigation into the physiology and biochemistry of the plant both prior to and after attack would seem essential in order to get a satisfactory solution of the ecological problems. Nutrition in regard to life history ought also to be studied. For instance, it would be interesting to know why the larva bores into the sapwood before pupating, and whether there is a constituent in this part essential for the mechanism of metamorphosis. The knowledge of wood-chemistry on the one side and of sense-physiology on the other must be more advanced before the question of attraction can be seriously gone into. Feeding experiments with artificial diet might also yield some more information of the principles underlying bark-beetle nutrition.

Summary

From investigations into the enzymes of the alimentary canal and a comparative analysis of ash bark and the frass of *Hylesinus fraxini* it appears that the food relations of the ash-bark beetle are as follows:

(1) Proteins are derived directly from the bark. The adult after emergence excretes more nitrogen than it consumes; this excess excretion may be regarded as representing waste-products of metamorphosis.

(2) The carbohydrates are derived from the following bark constituents:

(*a*) The simple sugars.

(*b*) Disaccharides, simple oligoses etc., probably all with α-linkages.

(*c*) The α-glucans up to starch, but with a definite preference for sugars of smaller chain-length. Starch as such has disappeared from the bark of attacked trees.

(*d*) The hexosan parts of the hemicelluloses.

(3) The protein-hydrolysing enzymes are of the tryptase and peptidase type, in accordance with the alkaline *p*H of the gut.

(4) The carbohydrate-hydrolysing enzymes are of the following types: saccharase, a weak lactase, maltase, amylase (dextrinase), galactanase.

(5) Cellulose is clearly not attacked. The same applies to all pentosans.

(6) The breakdown of starch in the attacked bark may serve as an attractant to the bark beetles, and may help to explain their preference for trees suffering from physiological or pathological disturbance.

(7) A way has been shown in which the hemicelluloses are utilized by the insect in question, and the probable fate of the breakdown products of these substances in the frass has been indicated.

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THE STEM AND BULB EELWORM, *ANGUILLULINA DIPSACI* (KUHN), IN STRAWBERRY IN BRITAIN

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(With Plate XVII)

Introduction

Anguillulina dipsaci has a known host range of some three hundred different plants but, owing to the presence within the species of numerous biologic strains or races, the host range of any particular strain is often strictly limited (Hodson, 1931). Widespread as the nematode is in Britain there is, apparently, no previous authentic record of its attacking strawberry here, and the main purpose of this paper is to record such occurrence. As the strawberry is already a well-known host plant in the United States, a brief discussion of the American literature is desirable.

Historical

An early account is that by McKay (1921) who records an attack at Cornwallis, Oregon, in 1916 upon cultivated strawberry and, subsequently (1922), upon wild strawberry in the same State. Later, Smith (1922) in Idaho writes: "The most obvious symptoms of the disease (*A. dipsaci*) on strawberry are enlargement and distortions of the stems, petioles and runners. Commonly the tops of flowering stems, petioles and leaves are swollen, dwarfed and abnormally shaped." Smith, while principally concerned with attack upon red clover, records an instance in which clover and strawberry upon adjacent land were both attacked. In one instance these crops overlapped; nevertheless, he could obtain no evidence that the nematodes could cross from the one to the other. Smith figures attacked strawberry plants and, making due allowance for varietal difference, his description and figures correspond closely with those symptoms observed in Britain.

As opposed to this, Godfrey (1934) describes and figures attacks upon *Fragaria chiloensis*, a wild strawberry, in the Pacific Northwest. His description of the injury caused differs markedly from that of Smith (1922) and, also, he found that he could readily transfer the nematodes from strawberry to red clover seedlings.

Finally, Courtney (1936) records and figures an attack upon strawberry, variety Marshall, following an attacked crop of red clover. Never-

theless, in this instance, the attack on strawberry resembles that described by Smith rather than that by Godfrey. Also, it should be noted that, in this instance, narcissus in the same land apparently escaped injury.

The foregoing accounts suggest that at least two, possibly three, biologic strains of the nematode, each with a different host range, have been observed upon strawberry in the United States. The following records indicate that more than one strain causes very similar injury in Britain.

OCCURRENCE IN BRITAIN

(1) *In the variety Madame Lefebvre* (*Pl. XVII, fig.* 1)

In 1934, the attention of the writer was drawn to a peculiar malformation of strawberry plants, variety Madame Lefebvre, occurring abundantly upon a commercial plantation in the Isle of Wight. Various "virus" diseases were present upon plants of other varieties growing in adjacent beds, and it was at first surmised that the malformation might be attributable to such infections. However, it was noted that upon some of the Madame Lefebvre plants, one or more crowns might be affected whilst others remained normal, a somewhat unlikely phenomenon if virus were responsible. Microscopic examination disclosed the invariable presence of *Anguillulina dipsaci* in affected plants. It was ascertained that parents of the plants had come originally from the mainland, and an examination of this parent stock disclosed a less severe but otherwise similar infection. This stock had originally been imported direct from the Continent and as the trouble, at least in 1934, was not found to be widely distributed in the variety elsewhere, it may be suggested that the plants were infected at the time of importation.

From 1934 until the time of writing (December 1937) this nematode infection has been continually under observation and some interesting facts have emerged. It appears that the strain of nematode concerned is a very highly specialized one. On the two commercial holdings on which the incidence has been followed closely, infestation has persisted throughout in the variety Madame Lefebvre, but has not spread to other varieties of strawberry and has not been found upon other cultivated plants or upon weeds. Infested plants removed from the Isle of Wight centre and established in the experimental plots at Reading have continued infested, and have passed infection on to their progeny but have entirely failed to infect strawberries of the varieties Royal Sovereign and Oberschlesien. This has remained true even when the latter varieties have been grown intermingled with infested plants and the whole bed

been allowed to produce runners indiscriminately, Madame Lefebvre runners only contracting an infection. Clover has not yet been subjected to infection and may, of course, prove to be an alternative host of the strain, but in 1935 and 1936 heavily infested Madame Lefebvre plants were grown over narcissus, varieties Soleil d'or and Golden Spur. In no case were the bulbs or foliage attacked or even entered by straying nematodes.

(2) *In the variety Royal Sovereign* (*Pl. XVII, fig.* 2)

Attack on this variety has been found only at Reading and has been referred to briefly by Buddin (1938). It was observed in two situations and, in each instance, the parent plants had been under observation for some time and could be ruled out as not providing a source of infection. Attack occurred in a series of plants grown in boxes and also in plants grown in an experimental plot. In each case it was definitely known that narcissi attacked by *A. dipsaci* had occupied the soil previously and no other probable source of infection could be found. At the outset the plants were entirely crippled by the attack, but in the course of time almost complete recovery took place. Now, upwards of 18 months after the initial infection occurred, symptoms are hard to find although the nematodes have, in fact, persisted in small numbers in some plants.

The inference to be drawn from the above is that at least one narcissus strain of the nematode is able vigorously to attack strawberry, variety Royal Sovereign, but that in the course of time the infestation tends to diminish. It must be recorded that the symptoms induced at the outset of these attacks were identical with those observed in the variety Madame Lefebvre, but in an infection experiment laid down recently, certain of the plants developed typical "Red Plant" symptoms, and further investigation of the problem is proceeding. Nevertheless, it certainly appears to be the case that several biologic strains of *A. dipsaci* can attack strawberry, and that the host ranges of the strains implicated here differ from those recorded from America.

Symptoms of attack

Leaves, petioles and flower stems are invaded. Affected leaves are brittle, curled, usually with the margins bent downwards, more or less crumpled, often somewhat thickened, particularly towards the junction with the petiole, basal portions of the main veins are somewhat thickened and the whole leaf frequently is extremely dwarfed. Petioles and flower stems are more or less thickened and shortened. In extreme cases the petioles are so shortened and the leaves so crumpled that the whole

crown forms a small tight rosette. In popular terms a typically affected leaf and petiole when removed from the plant superficially resembles closely a miniature stick of rhubarb. Although the leaf veins and petioles often exhibit somewhat uneven thickenings there is a marked absence of true "spikels" usually so typical of attack by this nematode and figured on wild strawberry by Godfrey & McKay (1934).

Commonly on a mature plant one or more crowns are badly affected while others may be to all appearances normal. Similarly an affected crown may throw rosette-like runners on thick swollen stolons, whilst other portions of the plant produce normal runners. The symptoms are most apparent in spring, autumn, and winter, and are liable to be masked to a considerable extent during the early summer when plants are growing freely.

ECONOMIC IMPORTANCE OF CONTROL MEASURES

As indicated previously, it appears that attacks by *A. dipsaci* on strawberry are not at present very widespread in this country, but that they occur at all gives cause for concern. Where observed on commercial holdings the depressing effect exercised, not only on the general vigour of the plantation, but also very definitely upon the cropping power of the plants has been apparent. Further, as strawberry plants are invariably propagated by vegetative means, there is every likelihood of infection being distributed elsewhere whenever runners are taken from affected plantations. In practice this cannot be overcome even by careful selection, for unless the runner at the time of planting has a fairly high nematode content, it will be unlikely at that time to exhibit any very marked symptoms of attack.

It is unlikely that plants can be freed from infection by the hot-water treatment. A strawberry plant cannot be relied upon to withstand, at a very maximum, more than 40 min. immersion in water at 110° F. This leaves only a small margin on the time taken to kill the nematode within a leaf or stem and, in practice, the treatment would probably be only partially successful. A small experiment carried out in 1936 confirmed this impression, surviving nematodes quickly again building up a large population within the plants.

In effect, with our present knowledge it can only be suggested that once the presence of *A. dipsaci* is diagnosed in a strawberry plantation, that plantation should on no account be used for runner production. Unless the attack is severe there is no great objection to leaving the plants in for the rest of their useful fruiting life, provided that precautions

against spreading infection are taken. At the termination of this period, some other crop should be substituted in the land for a few seasons.

As has been shown, if the attacked crop is the variety Madame Lefebvre the substitution of another variety of strawberry might meet the case. Unless, however, this is imperative it would be as well to select a less likely host, bearing in mind that clover and narcissus have, in some instances, proved acceptable alternatives to the nematode. In any case, predominant weeds in the locality should be carefully scrutinized as being possible host plants in which the nematodes might carry over until such time as strawberries are again planted in the land.

Summary

1. Attention is drawn to the occurrence of *Anguillulina dipsaci* on strawberry in Britain and reference is made to literature relating to such occurrence in the United States of America.

2. Recorded occurrences in Britain are discussed in some detail and reference is made to the host range of the strains of nematode implicated.

3. Symptoms of attack are described.

4. Control measures are discussed, the principal suggestion being that on no account should runners be taken for propagation from infected plantations.

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EXPLANATION OF PLATE XVII

Fig. 1. Madame Lefebvre, two-year-old plant. Attacked by *Anguillulina dipsaci* (strawberry strain). Contrast normal portion of plant with crumpled crown on right.

Fig. 2. Royal Sovereign, maiden plant. Attacked by *Anguillulina dipsaci* (narcissus strain). Contrast relatively healthy peripheral leaves with those around growing point.

(*Received* 14 *December* 1937)

Fig. 1

Photo A. Sherral

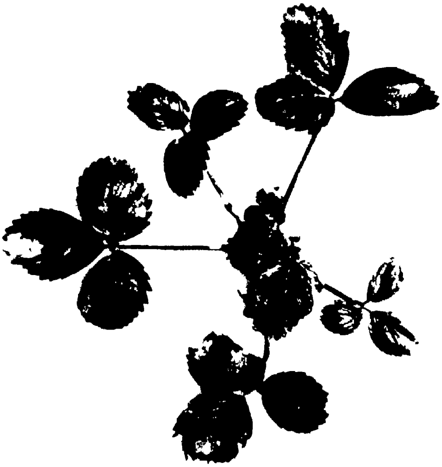

Fig. 2

Photo A. Sherral

HODSON.—THE STEM AND BULB EELWORM, *Anguillulina dipsaci* (KÜHN), IN STRAWBERRY IN BRITAIN (pp. 406–410)

THE PROBLEM OF THE EVALUATION OF ROTENONE-CONTAINING PLANTS

IV. THE TOXICITY TO *APHIS RUMICIS* OF CERTAIN PRODUCTS ISOLATED FROM DERRIS ROOT

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With an Appendix

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(With 2 Text-figures)

CONTENTS

Introduction

In Parts II and III of this series of papers (Martin & Tattersfield, 1936; Tattersfield & Martin, 1936) it was shown that there was a difference in the chemical make-up of the resins derived from the roots of the two species of *Derris*, *D. elliptica* and *D. malaccensis*. Thus the resins of the

former gave rise to little toxicarol when an ether solution was treated with alcoholic potash, whereas those of the latter were shown by chemical and polarimetric tests to be rich in a precursor which yielded toxicarol on subsequent treatment with alcoholic potash. This precursor was isolated in crystalline form (Tattersfield & Martin, 1937) and occurs in considerable amount in the Sumatra-type root; indeed, such roots are far richer in this product than they are in rotenone. Recently, also, Cahn & Boam (1935) have isolated from the Sumatra-type root a colourless crystalline derivative, which they have named sumatrol, and which they have shown to possess toxic properties. It is a matter of importance for evaluation purposes to know with some degree of accuracy how rotenone compares in toxicity with some of these isolated products, particularly with toxicarol, its precursor, with sumatrol and also with the resins derived from both *D. elliptica* and *D. malaccensis* when these have been freed as far as possible from crystalline material. The latter process must not be a drastic one, for there is now considerable evidence that certain of the crystalline products isolated from derris root have undergone change in the process of separation.

In the present work, two resins are dealt with, one derived from *D. elliptica* and one from *D. malaccensis* (Sumatra-type root). In the former the purified rotenone content was 8–9 %. In the process of separation no additional rotenone was added to aid its separation. It is a matter of doubt whether any of the processes at present employed for the separation of rotenone assures the removal of all present. The data given here, therefore, are necessarily a preliminary to further work on this subject.

The insecticidal tests were carried out by our usual procedure, using adult apterous females of *Aphis rumicis*. The experiments were made in fivefold replication, using five batches of ten insects for each concentration tested. The method adopted for comparing toxicity is based upon the work of Bliss (1935) and of Irwin (1937), a detailed example being given by Mr W. G. Cochran in the Appendix.

As the experiments have been spread over two seasons and the average susceptibility of the insects was not the same throughout the period, tests done on different dates cannot be expected to give the same absolute figures, but the comparative results for the toxicities relative to rotenone of different preparations of the toxicarol precursor are of the same order, the differences found not being significant.

The results indicate that of the known unaltered active principles of *Derris*, rotenone is the most toxic and therefore its determination in samples of derris root will always be necessary. As, however, both

the resins and such active principles as the toxicarol precursor and sumatrol may be present in certain strains of root in higher concentrations than rotenone, they cannot be neglected in assessing toxicity with any degree of accuracy. In addition, it seems doubtful whether the same toxic value can be placed upon the constituents left after the separation of rotenone in different strains of root.

EXPERIMENTAL

Preparation of the toxicarol precursor

In footnotes to Parts II and III of this series (*loc. cit.*) mention was made of an optically active crystalline compound that had been isolated from the ether solution of the Sumatra-type root by extraction with caustic potash. On the addition of small amounts of dilute caustic potash (2 %) to the ether solution of the Sumatra-type resin, a colloidal material was first extracted followed by a dark red clear solution on the addition of a further quantity of dilute alkali. On subsequently treating the ethereal layer with 5 % caustic potash, a copious bright yellow precipitate was deposited in the alkaline layer. On filtering, dispersing in water and acidifying in the presence of ether, a resin rich in toxicarol precursor was taken up by the ether. The ether solution after drying over anhydrous sodium sulphate deposited some yellow crystals which were filtered off and discarded. After concentrating and cooling in the refrigerator for some days a large mass of micro-crystalline matter was deposited. Repeated crystallizations from acetic acid or ethyl acetate raised the melting-point to 99° C. This product we have termed toxicarol precursor (potash separated).

As it is well known that the products derived from derris root are susceptible to the effects of alkali, it was considered advisable to attempt the separation of this product by direct means. This was finally effected by allowing a concentrated ethyl acetate solution of a Sumatra-type resin to evaporate slowly in the refrigerator. A small crop of crystals was obtained in this way, which proved of great value in seeding out larger quantities of the crude precursor from more dilute solutions of the resin. With material for seeding purposes, no difficulty presents itself in preparing this compound in a crystalline form. The controlled precipitation of the concentrated ether solution of the crude crystals with petroleum ether separated out first a darker coloured impure deposit, a further crop of relatively impure crystalline matter separating on cooling the supernatant liquid in the refrigerator after which the solution was concentrated in partial vacuum, cooled and the crystalline matter recrystal-

lized from ethyl acetate. The whole process was repeated several times. Repeated crystallizations raised the melting-point from 96 to 103° C. A brief account of these two derivatives has been published elsewhere (Tattersfield & Martin, 1937) but it is probable that even the material of higher melting-point is not completely pure.[1] With the material so far available, the following data have been accumulated:

Toxicarol precursor (potash separated).
Melting-point 99–100° C.
$[\alpha]_D^{20} = -68{\cdot}8°$ in benzene, concentration 4·0536 g. per 100 ml.
$[\alpha]_D^{20} = -71{\cdot}2°$ in benzene, concentration 2·0268 g. per 100 ml.
$[\alpha]_D^{20} = +41{\cdot}5°$ in ethyl alcohol, concentration 1·0008 g. per 100 ml.

Toxicarol precursor (direct separation).
Melting-point 96° C.
$[\alpha]_D^{20} = -66{\cdot}8°$ in benzene, concentration 4·0009 g. per 100 ml.
$[\alpha]_D^{20} = -70{\cdot}9°$ in benzene, concentration 2·0044 g. per 100 ml.
Melting-point 95° C.
$[\alpha]_D^{20} = -66{\cdot}8°$ in benzene, concentration 4·0184 g. per 100 ml.
$[\alpha]_D^{20} = -69{\cdot}7°$ in benzene, concentration 2·0092 g. per 100 ml.
Melting-point 102–103·5°.[1]
$[\alpha]_D^{20} = -67{\cdot}6°$ in benzene, concentration 4·3168 g. per 100 ml.
$[\alpha]_D^{20} = +44{\cdot}8°$ in ethyl alcohol, concentration 1·0044 g. per 100 ml.
$[\alpha]_D^{20} = +41{\cdot}5°$ in ethyl alcohol, concentration 1·0136 g. per 100 ml.
$[\alpha]_D^{20} = +41{\cdot}8°$ in ethyl alcohol, concentration 0·7174 g. per 100 ml.

Change in sign of rotation

The precursor separated either by potash treatment or by direct crystallization shows the change over in the rotation of its benzene solution when caustic potash in methyl alcohol is added. The subsequent decline in rotation proceeds at approximately the same speed as that found for the Sumatra-type resin and for the potash-extracted resin, when the amount of alcohol added and the temperature of the reaction are the same. Progressive darkening of the solution renders readings of the instrument very difficult after some time and finally impossible, but as far as could be judged the fall in rotation does not proceed to zero,

[1] Cahn (private communication) has confirmed the presence of this precursor in the resins of the Sumatra-type root, and has given much attention to its purification and constitution. The purification of this derivative is one of great difficulty and purity cannot be judged by melting-point considerations. Our product, in his view, contains almost certainly a proportion of sumatrol and the true rotation of the precursor is definitely lower than the figures given.

possibly indicating the presence of some other material. When the final reading is deducted, the rotations follow an approximately unimolecular curve. The coefficient $k = \frac{1}{t_2 - t_1} \log \frac{a}{a-x}$, however, falls off slowly, but during the middle period of the reaction is nearly constant.

The following is a summary of the results:

Toxicarol precursor (potash separated).

1 g. equivalent of caustic potash in methyl alcohol added, the molecular weight being regarded as the same as toxicarol.

Concentration of benzene solution 2·2027 g. per 100 ml.

Proportion of benzene solution : methyl alcohol :: 5 : 2.

$[\alpha]_D^{20}$ for benzene solution $= -71{\cdot}2°$.

$[\alpha]_D^{20}$ after adding methyl alcohol $= -31{\cdot}7°$.

On adding 1 g. equivalent methyl alcoholic potash:

$[\alpha]_D^{20}$ after 2 min. $= +302{\cdot}5°$.

$[\alpha]_D^{20}$ after 15 min. $= +236°$.

$[\alpha]_D^{20}$ after 120 min. $= +120°$.

$[\alpha]_D^{20}$ after 360 min. $= +56°$.

After 23 hr. an accurate reading could not be taken but $[\alpha]_D^{20}$ was not lower than $+52°$.

Mean k after deducting $[\alpha]_D^{20}$ for $t = 360$ min. for intervals

$t = 13$ min. to $t = 118$ min. $= 0{\cdot}0095$ to log base 10

$= 0{\cdot}022$ to log base e.

Toxicarol precursor by direct separation.

1 g. equivalent of caustic potash added in methyl alcohol.

Concentration of benzene solution 2·0092 g. per 100 ml.

Proportion of benzene solution : methyl alcohol :: 5 : 2.

$[\alpha]_D^{20}$ for benzene solution $= -69{\cdot}7°$.

$[\alpha]_D^{20}$ after adding methyl alcohol $= -33{\cdot}4$.

On adding methyl alcoholic caustic potash:

$[\alpha]_D^{20}$ after 2 min. $= +285°$.

$[\alpha]_D^{20}$ after 15 min. $= +222{\cdot}3°$.

$[\alpha]_D^{20}$ after 120 min. $= +55{\cdot}7°$.

$[\alpha]_D^{20}$ after 360 min. $= +35{\cdot}5°$.

$[\alpha]_D^{20}$ after 1560 min. $= +32{\cdot}2°$.

Mean k after deducting $[\alpha]_D^{20}$ for $t = 1560$ min. for intervals

$t = 13$ min. to $t = 88$ min. $= 0{\cdot}0093$ to log base $10 = 0{\cdot}0021$ to log base e.

The data from two examples out of several tests are plotted in Fig. 1, those for the potash separated precursor (M.P. 99° C.) being shown in

section A, and for the compound separated directly (M.P. 96–98° C.) in section B.

The results are expressed in each section in two ways. The times which have elapsed from adding the caustic potash in methyl alcohol to the respective benzene solutions are plotted against (*a*) the specific rotation, (*b*) the logarithms of the figures given by deducting the last observable specific rotation from each of the others. There is a slight difference in the results for the two products probably due either to differences in purity, or to difficulties found in controlling the temperature, particularly in the initial stages of the reaction.

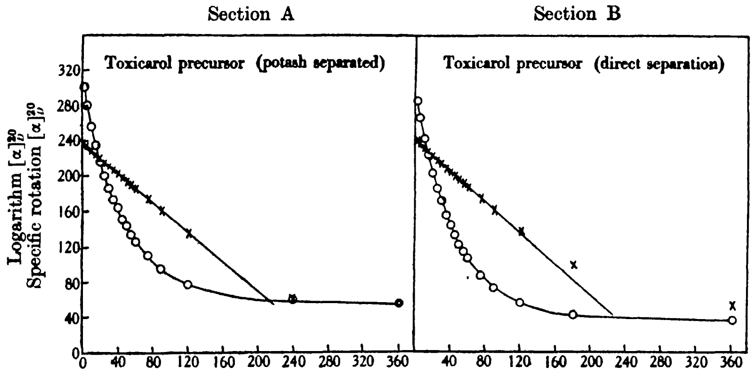

Fig. 1. Decline in specific rotation of toxicarol precursor in benzene solution after the addition of caustic potash in methyl alcohol. ○ ○ Specific rotations $[\alpha]_D^{20}$; × × $\log_{10}$ specific rotations $[\alpha]_D^{20}$.

It is clear, however, from the close parallelism of the semi-logarithmic data graphed in the two sections of Fig. 1 that they each contain a very high proportion of the same active constituent.

Tests were also carried out with solutions in ethyl alcohol of the compound isolated by direct crystallization, and melting 102–103·5° C. In one case 1 g. equivalent and in the other 4 g. equivalents of caustic potash in ethyl alcohol were added. Owing to the rapidity of the fall in optical activity and the relatively rapid darkening of the solutions the results were not very satisfactory. The results are summarized below.

First concentration of the alcoholic solution 1·0044 g. per 100 ml. $[\alpha]_D^{20} = +44{\cdot}8°$.

Concentration, after adding ethyl alcohol equal in amount to that introduced with the caustic potash, $=0{\cdot}7174$ g. per 100 ml. $[\alpha]^{0} = +\ 41{\cdot}8°$

On adding 1 g. equivalent of caustic potash in methyl alcohol.

$[\alpha]_D^{20}$ after 2 min. = +166°.

$[\alpha]_D^{20}$ after 5 min. = +89°.

$[\alpha]_D^{20}$ after 90 min. = +56°.

On adding 4 g. equivalent of caustic potash in same volume of alcohol $[\alpha]_D^{20}$ after 1 min. = +293°.

$[\alpha]_D^{20}$ after 2 min. = +248°.

$[\alpha]_D^{20}$ after 5 min. = +157°.

$[\alpha]_D^{20}$ after 114 min. = +46°.

The value $\frac{1}{t_2 - t_1} \log \frac{a}{a-x}$ was not constant.

INSECTICIDAL TESTS

Preparation of spray fluids

Rotenone. Rotenone was separated as the carbon tetrachloride complex and recrystallized several times from absolute alcohol, the product being finally dried in a vacuum desiccator. A weighed quantity was dissolved in absolute alcohol and made up to a known concentration. Dilutions were made with 0·5 % saponin solution and each dilution adjusted to contain the same proportion of alcohol. The dilution for each concentration used was made just prior to spraying, not more than a few minutes elapsing between the addition of the saponin solution and the completion of the five replications. Thus the risk of aggregation of particles was minimized and no separation of a deposit was noted.

Toxicarol precursor preparations. These included: (1) The micro-crystalline material isolated from the Sumatra-type resin, by extracting the ether solution of the latter with caustic potash and recovering the free compound from the potash-precipitated material by acidification and extraction. (2) The crystals obtained from the latter by crystallization from glacial acetic acid. It was a matter of some difficulty to free the product from the last traces of acetic acid; it was therefore thoroughly washed with cold alcohol, and the last traces of solvent taken off *in vacuo*. (3) The micro-crystalline crude product isolated was frequently recrystallized from ethyl acetate. (4) The precursor separated by direct crystallization from a sample of the Sumatra-type resin was subjected to the process of purification outlined on p. 413 and finally recrystallized several times from ethyl acetate (M.P. 102–103·5° C.).

For the insecticide trials, a solution in alcohol of known strength was diluted in precisely the same way as in the case of rotenone.

Inactive toxicarol. The precursor dissolved in ethyl alcohol was refluxed with a small amount of aqueous caustic potash, allowed to stand for an hour, the deposit filtered off, washed with boiling absolute alcohol, dried at 60–80° C. for half an hour and recrystallized from acetic acid. As toxicarol is relatively insoluble in alcohol, it was dissolved in benzene and emulsions of different concentrations were prepared by the addition of 0·5 % saponin just before spraying. Under these conditions the product showed little or no toxicity at 0·5 % concentration.

Sumatrol. The crystals were dissolved in acetone, and the appropriate dilutions made just before spraying with 0·5 % saponin solution in water, the amount of acetone being adjusted to the same content in each.

Resin from the Sumatra-type root freed as far as possible from crystallizable material. The ether extract of a large quantity of the root was transferred to a large separating funnel and extracted successively with four lots of aqueous potash (5 %). The extracted ether solution was thoroughly washed with water and dried over sodium sulphate, the ether removed and the residue dissolved in carbon tetrachloride and cooled in a refrigerator for 4 days. The rotenone crystallized and was filtered off. The solvent in the filtrate was taken off under reduced pressure with warming, in a vacuum desiccator, and finally at 100° C. under reduced pressure. Dilutions for spraying purposes were made from an alcohol solution of known content precisely as in the case of rotenone.

Resin from Derris elliptica. The ether extract was dissolved in carbon tetrachloride and cooled in a refrigerator for many weeks, the rotenone filtered off, and the filtrate cooled again. After the separation of a further small amount of crystalline matter, the solvent was taken off as with the Sumatra-type resin and dilutions for spraying purposes made as for rotenone from an alcohol solution of known content of resin.

Results

Adult apterous females of *Aphis rumicis* were used as test subjects with the usual technique for spraying and assessing the results. Ten insects at a time were sprayed and five replications were made. The insects after being sprayed were kept in muslin-covered tubes in a dark room of relatively constant temperature, examined after 2 days (44 hr.), and separated into five categories: (1) the unaffected, (2) those slightly affected, (3) the seriously paralysed (*B*), (4) the moribund (*M*), and (5) the apparently dead (*D*). Since in the last three categories there is rarely, if ever, any recovery from the effects of these compounds, the number

Table I

Relative potencies of rotenone and certain constituents of Derris

Insect used *Aphis rumicis*. Sprayed in fivefold replication 10 insects at a time. b_c = mean slope of regression lines, $V(b_c)$ its variance, $\Sigma(w_1)$ and $\Sigma(w_2)$ = sum of weights, s_M^2 = variance of M.

Relative potencies (antilog M)

1. $M = \bar{x}_1 - \bar{x}_2 + \frac{\bar{y}_2 - \bar{y}_1}{b_c}$
2. S.E.$_M = \sqrt{s_M^2} = \sqrt{\frac{1}{b_c^2}\left\{\frac{1}{\Sigma(w_1)} + \frac{1}{\Sigma(w_2)} + \frac{(\bar{y}_2 - \bar{y}_1)^2}{b_c^2} V(b_c)\right\}}$
3. S.E. of relative potency $= \log_e 10 \times$ antilog $M \times s_M$

Conc. mg./litre	Log conc. x	Paralysed and dead insects % allowing for control	Probits y	No. of insects used	Relative potencies (antilog M)
Series 1. Section A of Fig. 2.					
Toxicarol precursor (potash separated) (microcrystalline):					
400	2·602	97·9	7·034	49	
200	2·301	67·3	5·448	50	
100	2·000	14·9	3·959	48	$M = 1·1638$
75	1·875	4·0	3·249	50	S.E.$_M = \pm 0·0358$
					Potency of rotenone / Potency of toxicarol precursor = antilog M = 14·6
Rotenone:					
40	1·602	100	8·232*	51	S.E. of relative potency $= \pm 1·2$
20	1·301	90	6·282	51	
10	1·000	39·5	4·734	49	
5	0·699	4·7	3·325	45	
Control		2·1		47	
Series 2. Section B of Fig. 2.					
Toxicarol precursor (potash separated) (crystallized from acetic acid):					
400	2·602	77·5	5·755	51	
300	2·477	66·7	5·432	50	
200	2·301	30·5	4·490	51	$M = 1·1863$
150	2·176	6·25	3·466	50	S.E.$_M = \pm 0·0287$
					Potency of rotenone / Potency of toxicarol precursor = antilog M = 15·4
Rotenone:					
30	1·477	89·4	6·248	49	S.E. of relative potency $= \pm 1·0$
20	1·301	72·9	5·610	50	
15	1·176	29·8	4·470	49	
Control		4·0		50	
Series 3. Section C of Fig. 2.					
Toxicarol precursor (direct separation), M.P. 102–103·5:					
					$M = 1·1629$
301	2·479	97·9	7·033	50	S.E.$_M = \pm 0·0398$
201	2·303	74·4	5·656	50	Potency of rotenone / Potency of this sample of precursor = antilog M = 14·6
100·4	2·002	25·2	4·332	50	S.E. of relative potency $= \pm 1·33$
Toxicarol precursor (potash separation) (crystallized from ethyl acetate):					
					$M = 1·1137$
300	2·477	95·7	6·717	50	S.E.$_M = \pm 0·0420$
200	2·301	80·8	5·870	50	Potency of rotenone / Potency of this sample of precursor = antilog M = 13·0
100	2·000	37·2	4·673	51	S.E. of relative potency $= \pm 1·26$

* Computed value.

Table I (*cont.*)

Relative potencies (antilog M)

1. $M = \bar{x}_1 - \bar{x}_2 + \frac{\bar{y}_2 - \bar{y}_1}{b_c}$

2. $\text{S.E.}_M = \sqrt{s_M^2} = \sqrt{\frac{1}{b_c^2}\left\{\frac{1}{\Sigma(w_1)} + \frac{1}{\Sigma(w_2)} + \frac{(\bar{y}_2 - \bar{y}_1)^2}{b_c^2} V(b_c)\right\}}$

3. S.E. of relative potency $= \log_e 10 \times \text{antilog } M \times s_M$

Conc. mg./litre	Log conc. x	Paralysed and dead insects % allowing for control	Probits y	No. of insects used	Relative potencies (antilog M)
Series 3 (*cont.*)					
Sumatrol:					
404	2·606	100	7·338*	50	$M = 1{\cdot}1179$
302	2·480	92·7	6·454	50	$\text{S.E.}_M = \pm 0{\cdot}0454$
201	—	48·8†	—	50	$\frac{\text{Potency of rotenone}}{\text{Potency of sumatrol}} = \text{antilog } M = 13{\cdot}1$
100·6	2·002	37·8	4·689	49	S.E. of relative potency $= \pm 1{\cdot}37$
Rotenone:					
31	1·491	100	7·506	50	
20·7	1·316	91·5	6·372	50	
10·4	1·017	53	5·075	50	
5·2	0·716	19·3	4·133	49	
Control		6·4		47	
Control for sumatrol		18		50	
Series 4. Section D of Fig. 2.					
Sumatra-type resin (separated from rotenone and toxicarol precursor):					
300	2·477	100	7·966*	49	
200	2·301	100	7·035*	48	
100	2·000	35·4	4·626	49	$M = 0{\cdot}800$
80	1·903	8·6	3·634	48	$\text{S.E.}_M = 0{\cdot}0247$
60	1·778	9·1	3·665	46	$\frac{\text{Potency of rotenone}}{\text{Potency of this resin}} = \text{antilog } M = 6{\cdot}3$
					S.E. of relative potency $\pm 0{\cdot}36$
Rotenone:					
20	1·301	61·7	5·298	48	
15	1·176	33·4	4·571	49	
Control		2·0			
Series 5. Section E of Fig. 2.					
Derris elliptica resin (W. 186) (separated from rotenone):					
100·5	2·002	100	7·679*	50	
80·4	1·905	100	7·190*	50	
60·3	1·780	84·1	5·999	50	$M = 0{\cdot}5515$
40·2	1·604	50	5·000	50	$\text{S.E.}_M = \pm 0{\cdot}0311$
					$\frac{\text{Potency of rotenone}}{\text{Potency of this resin}} = \text{antilog } M = 3{\cdot}6$
Rotenone:					S.E. of relative potency $= \pm 0{\cdot}26$
20·7	1·316	97·7	6·995	50	
10·4	1·017	35·1	4·617	49	
Control		12·0			
Series 6. Section F of Fig. 2.					Results omitting value in brackets for rotenone
D. elliptica resin (W. 186) (separated from rotenone):					
100	2·000	100	7·696*	47	$M = 0{\cdot}5653$
80	1·903	100	7·296*	49	$\text{S.E.}_M = \pm 0{\cdot}03225$
60	1·778	91·2	6·353	49	$\frac{\text{Potency of rotenone}}{\text{Potency of this resin}} = \text{antilog } M = 3{\cdot}7$
40	1·602	55·9	5·148	46	
20	1·301	15·9	4·001	47	S.E. of relative potency $= \pm 0{\cdot}27$
Rotenone:					Results taking value in brackets for rotenone
31	1·491	100	8·168*	49	$M = 0{\cdot}5896$
20·7	1·316	97·9	7·033	50	$\text{S.E.}_M = \pm 0{\cdot}0284$
(15·5	1·190	95·6	6·706	49)	$\frac{\text{Potency of rotenone}}{\text{Potency of this resin}} = \text{antilog } M = 3{\cdot}9$
10·4	1·017	56·4	5·161	49	
7·8	0·892	39·6	4·736	39	S.E. of relative potency $= \pm 0{\cdot}25$
Control		6·4			

* Computed value. † Value omitted from calculation as it introduces heterogeneity.

falling within them were listed together for judging toxicity $(B+M+D)$. Occasionally during spraying an insect was lost or in the examination a diseased insect had to be eliminated, accounting for occasional discrepancies from the figure 50 shown in the tables.

The data are shown in Table I. The concentrations and their logarithms (x), the percentage of seriously affected insects with the corresponding probits and the actual numbers of insects used for assessment and determining the "weights" to be allotted are given. In addition, the result of each experiment is shown in the last column. In

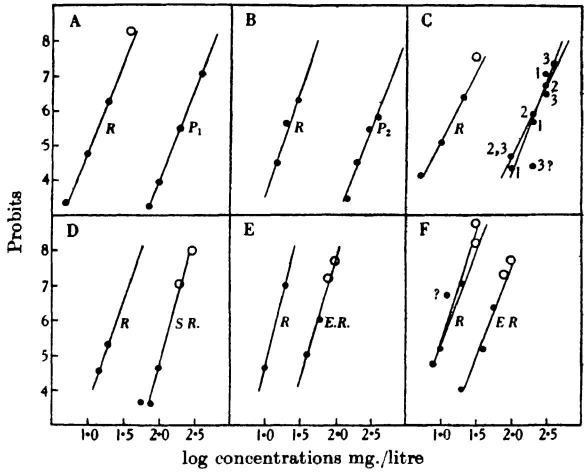

Fig. 2. Probits plotted against log concentrations for rotenone, and various constituents of derris root. ○ computed, R rotenone, 1 toxicarol precursor, direct separation, 2 toxicarol precursor, potash separation, 3 sumatrol, $S.R.$ rotenone-freed Sumatra resin, $E.R.$ rotenone-freed *D. elliptica* resin, P_1 micro-crystalline precursor separated from ether, P_2 recrystallized acetic acid.

Fig. 2 the probits are plotted against the logarithms of the concentrations, the lines drawn through the points being calculated. There was on the whole little difference in position between the free-hand lines used for assessing the "weights" described by Bliss (1935) and the calculated lines, and since the scale of plotting is small, only the latter are shown in order to avoid confusion.

The method of statistical analysis is somewhat laborious, but has proved in our experience to be of considerable value. Its use and particularly the test for the heterogeneity makes it possible to eliminate unreliable values, due to unavoidable imperfections in the process of

carrying out a large-scale laboratory spraying experiment. Such points have been eliminated from the data. In addition, it was noted that above certain levels of concentration there was a decline in toxicity. We have observed this effect with derris preparations over a period of years. The most valid explanation appears to be that the result is due to aggregation of particles in the emulsions or possibly in some cases to the formation of crystals above an optimum size, since it was shown by Fryer *et al.* (1923) that the toxicity of rotenone depended upon the size of the crystals. Therefore, in order to eliminate this effect as far as possible, dilutions of the concentrated extracts were made in every case just before spraying.

Bliss (1935) has also demonstrated that frequently in these types of data there is, for small probit values, a departure from the general linearity obtained at higher concentrations. This was not a noticeable feature of our data, but in one or two cases at the lowest concentrations tested there was some indication of this break and these values have not been included in the analyses.

In one case (Table I, series 3 and Fig. 2, section 3), heterogeneity was introduced into the data by one determination of toxicity unquestionably faulty and this was eliminated. The point is shown with a query mark. In section F, Fig. 2, a point of questionable validity also appears, but as it does not introduce heterogeneity we have calculated the relative potencies in this case, both by leaving it out, and taking it into account. The results, however, are not significantly different, and both regression lines are given in section F.

In section C the slope of the line for the toxicarol precursor (direct separation) is slightly steeper than that representing the potash-separated precursor and sumatrol, but as neither line differs significantly from that of rotenone in parallelism this difference may be regarded as of no importance. The data for sumatrol are not entirely satisfactory, one experimental value being obviously an under-estimate. We do not wish therefore to put any great stress upon the determination of the relative potency of this compound, as owing to its low solubility in alcohol, acetone was used in the preparation of the spray and the control test, in which an equivalent amount of acetone was used, led to the relatively high figure of 18 % of paralysed insects. The results, however, indicate that sumatrol is of the same order of toxicity to *Aphis rumicis* as the toxicarol precursor. Toxicarol itself when sprayed in benzene emulsions had little or no toxicity at 0·5 % concentration.

The relative toxicities given by comparisons at the median lethal

doses approximately computed from the free-hand regression lines, together with those determined statistically are shown in Table II.

Table II

Comparison of estimates of relative potencies computed from free-hand probit-log-concentration lines at probit 5·0 *and by statistical analysis*

Results expressed as $\frac{\text{Potency of rotenone}}{\text{Potency of substance tested}}$.

Section in Fig. 2	Rotenone compared with	Log conc. of rotenone at probit 5·0	Log conc. of substance tested at probit 5·0	Difference	Relative potency computed from free-hand lines at probit 5·0 antilog of difference	Relative potency from statistical computation
A	T. precursor (KOH separated microcrystalline)	1·045	2·210	1·165	14·6 : 1	14·6 : 1
B	T. precursor (KOH separated crystallized acetic acid)	1·235	2·405	1·170	14·8 : 1	15·4 : 1
C	T. precursor (KOH separated crystallized ethyl acetate)	0·978	2·085	1·107	12·8 : 1	13·0 : 1
C	T. precursor (direct separation)	0·978	2·165	1·187	15·4 : 1	14·6 : 1
C	Sumatrol*	0·978	2·150	1·172	14·9 : 1	13·1 : 1
D	Sumatra-type resin	1·250	2·060	0·810	6·5 : 1	6·3 : 1
E	*Derris elliptica* resin (1)	1·065	1·610	0·545	3·5 : 1	3·6 : 1
F	*Derris elliptica* resin (2)†	0·965	1·525	0·560	3·6 : 1	3·7 : 1

* A discrepant point taken in. † A discrepant point omitted.

The figures from the free-hand lines were obtained by taking the lines used for computing the weights in the statistical analyses and reading off the respective log concentrations corresponding to 5·0 probits, subtracting and reading the antilog. The differences between the relative potencies by the two methods are not great, and are not significantly different from each other. The shorter method, however, does not permit determination of the standard error and unless the points through which it is drawn are disposed in such a way as to be easily fitted by eye, a considerable degree of error may result. Such a method, however, does give a very useful preliminary estimate and in many cases may be all that is required.

It may be noted that the potencies of the samples of the toxicarol precursor are not significantly different from each other. Sumatrol also, in our experiments has the same order of toxicity to *A. rumicis* as the toxicarol precursor, and although our samples of the precursor may

contain a certain proportion of sumatrol, the indications are that this order of toxicity would be shown by the pure precursor unless a highly potent but as yet unrecognized compound is also present.

The rotenone and toxicarol-free resin of the Sumatra-type root and the rotenone-free resin of *Derris elliptica* are more toxic than either the toxicarol precursor or sumatrol, but the resin derived from the Sumatra-type is less toxic than that derived from *D. elliptica*. Rotenone was six times as toxic as the Sumatra-type resin and four times that derived from *D. elliptica*. Rotenone was not in either case added as an aid to its own separation, but it is reasonable to believe that the presence of rotenone in the resins would, if anything, increase toxicity, and the deduction can be drawn that the suggestion of Jones & Smith (1936) to evaluate the residual rotenone-free resins as possessing half the toxic value of rotenone is scarcely justified as far as *Aphis rumicis* is concerned. Jones & Smith, however, used house flies for the determination of their toxicities, and it is possible that the degree of difference may depend on the test-subject used.

Conclusions

Previous papers in this series (*loc. cit.*) have indicated a difference in the chemical constitution of the resins extracted from roots of *Derris elliptica* and *D. malaccensis*. In this paper it is shown that a crystalline derivative giving rise to toxicarol on treatment with alcoholic potash and which may be regarded as its precursor, is largely responsible for the chemical and physical properties which differentiate the Sumatra-type and *D. malaccensis* resins from those of *D. elliptica*. This compound is readily isolated in large amounts from the potash extraction of ether solutions of Sumatra-type resin or by direct crystallization from the latter, and is responsible for the characteristic change in sign of rotation of the benzene solutions of the resins of *D. malaccensis* on addition of alcoholic potash. It is laevo-rotatory in benzene and dextro-rotatory in alcohol. After the addition of caustic potash in alcohol to either its benzene or alcoholic solutions, the dextro-rotatory power declines with time.

The first preparations by both the above processes melted in the neighbourhood of 97–100° C. The melting-point of the directly separated compound has been raised by repeated crystallizations and precipitations using various solvents to the neighbourhood of 103° C., but the product may still contain allied compounds.

Rotenone is fourteen to fifteen times as toxic as our present preparations of toxicarol precursor. The Sumatra-type resin from which both the precursor and rotenone have been separated is also more toxic, nevertheless the precursor in our tests possesses much more insecticidal power than inactive toxicarol, and in the Sumatra-type root almost certainly contributes in a large measure to its toxicity to insects.

This paper is mainly devoted to the description of toxicity tests made with this compound or, perhaps, complex of compounds, and with the resins freed from rotenone as far as possible without the addition of the latter compound to aid its own separation, rotenone being used as a standard of comparison. An experiment carried out with sumatrol is also included. An Appendix by Mr W. G. Cochran describes the most suitable and least laborious statistical technique applicable to the analysis of our results and to the determination of their significance. A rough method of comparing toxicities by reading directly from the probit-log concentration lines was also employed. We find that the toxicities to *Aphis rumicis* of the toxicarol precursor and of sumatrol are of the same order, about one-thirteenth to one-fifteenth as toxic as rotenone, whereas the resin of the Sumatra-type root is about one-sixth and that of *Derris elliptica* about one-fourth as toxic as rotenone.

These data suggest that one factor for purposes of evaluation cannot be applied to all classes of root, irrespective of their derivation. Thus, it would appear at present unjustifiable to assume that the resins of different varieties, after the separation of rotenone by present methods, have a toxicity of a definite and fixed order, or, until further work has been done on the relationship between the rotation of the various naturally occurring active principles and their toxicities, that a determination of the rotations of extracts derived from different varieties of root will give a valid assessment of their insecticidal value.

We wish to express our indebtedness to the Cooper Technical Bureau for a gift of sumatrol, to Dr R. S. Cahn for permission to publish a short private communication, and to Mr W. G. Cochran for statistical help and advice.

APPENDIX

By W. G. COCHRAN

The following is a detailed example of the computation necessary for determining the relative potencies. The figures are taken from Table I, series 1 (p. 419).

	Log conc. x	Probits y	Weight w	wx	wy
Rotenone	1·602	8·232	0·918	1·470636	7·556976
	1·301	6·282	16·269	21·165969	102·201858
	1·000	4·734	30·429	30·429	144·050886
	0·699	3·325	8·100	5·6619	26·9325
		Total	55·716	58·727505	280·742220
Toxicarol precursor	2·602	7·034	6·958	18·104716	48·942572
	2·301	5·448	29·550	67·994550	160·988400
	2·000	3·959	19·968	39·936	79·053312
	1·875	3·249	10·400	19·5	33·789600
		Total	66·876	145·535266	322·773884

Notes		Rotenone (r)	Toxicarol precursor (p)
(i)	$\bar{x}=\dfrac{\Sigma\,(wx)}{\Sigma w}$	1·054051	2·176196
	$a=\bar{y}=\dfrac{\Sigma\,(wy)}{\Sigma\,(w)}$	5·038808	4·826453
(ii)	$A=XX=\Sigma\,(wx^2)-\bar{x}\Sigma\,(wx)$	2·3778	3·2852
	$XY=\Sigma\,(wxy)-\bar{x}\Sigma\,(wy)$	12·0310	16·8258
	$YY=\Sigma\,(wy^2)-\bar{y}\Sigma\,(wy)$	61·1224	86·2283
	(Slope of regression line) $b=\dfrac{XY}{XX}$	5·0597	5·1217
	$\chi^2=YY-b\,(XY)$	0·249	0·052

χ^2 for $n=n'-2=2$ and $P=0{\cdot}05$ is 5·991, $\therefore$ data may be regarded as not heterogeneous

		Rotenone (r)	Toxicarol precursor (p)
(iii)	Variance of a: $V(a)=1/\Sigma\,(w)$	0·01795	0·01495
	Variance of b: $V(b)=1/A=\dfrac{1}{XX}$	0·4206	0·3044

Test for parallelism:

$$\chi_b{}^2=\frac{(b_r-b_p)^2}{\dfrac{1}{A_r}+\dfrac{1}{A_p}}=0{\cdot}005$$

For $n=1$, $P=0{\cdot}05$, $\chi^2=3{\cdot}481$, departure of lines from parallelism not significant

Determination of relative potencies:

$$M=\log\left(\frac{\text{potency of rotenone}}{\text{potency of toxicarol precursor}}\right)=\bar{x}_p-\bar{x}_r+(\bar{y}_r-\bar{y}_p)/b_c$$

Mean slope of combined lines: $b_c=\{(XY)_r+(XY)_p\}/\{(XX)_r+(XX)_p\}$
$=5{\cdot}0957$

$$V\,(b_c)=\frac{1}{A_r+A_p}=\frac{1}{(XX)_r+(XX)_p}=0{\cdot}17658$$

(iv) $M=1{\cdot}1221+0{\cdot}04167=1{\cdot}1638$

Relative potency: Antilog $M=14{\cdot}58$

(v) *Standard error of M (s_M):*

$$s_M=\sqrt{V\,(M)}=\frac{1}{b_c}\sqrt{\left[1/\Sigma\,(w_r)+1/\Sigma\,(w_p)+\frac{(\bar{y}_r-\bar{y}_p)^2}{b_c^2}\,V\,(b_c)\right]}=\pm 0{\cdot}03576$$

(vi) *S.E. of relative potency:* $\log_e 10\times\text{antilog}\,M\times s_M=2{\cdot}3026\times 14{\cdot}58\times 0{\cdot}0358=\pm 1{\cdot}20$

The object of the calculation is to obtain, with the least possible labour, the value of M correct to three decimal places. The specimen calculation includes all the figures that need be written down in machine computations and shows the number of decimal places that should be retained at each stage of the calculation. The column of products, wx and wy, should first be formed; no rounding-off is recommended here. These figures serve a double purpose; they give by summation the values of $\Sigma\,(wx)$, $\Sigma\,(wy)$ and hence $\bar{x}$ and $\bar{y}$, and they facilitate the calculation of $\Sigma\,(wx^2)$, etc.

(i) $\bar{x}$ and $\bar{y}$ should be calculated to six decimal places. They will be multiplied by about 281 and 323 respectively in finding XY and YY, and the latter are required to be correct to four decimal places.

(ii) $\Sigma\,(wx^2)$ is obtained by taking the sum of products of wx and x. The correction term $\bar{x}\Sigma\,(wx)$ can be subtracted on the machine, since $\bar{x}$ and $\Sigma\,(wx)$ have already been written down.

(iii) These expressions give, by taking the square root, the standard errors of a and b if these are of interest; the expressions are also required later in the calculation.

(iv) The expressions $(\bar{x}_p-\bar{x}_r)$ and $(\bar{y}_r-\bar{y}_p)/b_c$ need not be written down separately for the calculation of M, but the latter is useful for the calculation of s_M.

(v) To calculate s_M without writing down any intermediate figures, first square $\left(\frac{\bar{y}_r-\bar{y}_p}{b_c}\right)=0{\cdot}04167$. Multiply the answer by $V\,(b_c)$ and add the other two terms under the square root. The square root is then found, put on the machine, and divided by b_c to give s_M. Irwin uses the notation σ_M for s_M. The use of s_M, however, accords with the general convention in statistics that Roman letters refer to estimates and Greek letters to population values.

(vi) The formula given for the standard error of the relative potency (antilog M) is an approximation, which is quite satisfactory when, as in this example, s_M is small compared with M.

Two tests of significance may be wanted: (1) to test whether the potencies of the two poisons which are being compared in a single experiment are significantly different, i.e. whether the relative potency is significantly different from unity; (2) to test the significance of the difference between the relative potencies of two poisons which have been compared, in different experiments, with the same control. Both these tests should be made in the log scale.

To test whether M differs significantly from zero, we may regard s_M

(as derived in the specimen calculation) to be based on (n_1+n_2+1) degrees of freedom, where n_1, n_2 are the numbers of degrees of freedom in the χ^2 tests for homogeneity of the two poisons. In the present example, $n_1=n_2=2$. This test is sufficiently accurate if $t^2V(b_c)/b_c^2$ is small compared with unity, where t is the value of Student's t, for (n_1+n_2+1) degrees of freedom, at the level of significance desired. In the present example, at the 5% level, $t=2{\cdot}571$ and $t^2V(b_c)/b_c^2$ is 0·045.

The exact test of significance will rarely be required, but may be made by regarding the relative potencies as significantly different if

$$\bar{x}_2-\bar{x}_1+\frac{(\bar{y}_1-\bar{y}_2)}{b_c(1-\lambda)}-\frac{t}{b_c(1-\lambda)}\sqrt{\{1/\Sigma(w_1)+1/\Sigma(w_2)\}(1-\lambda)+\frac{(\bar{y}_2-\bar{y}_1)^2V(b_c)}{b_c^2}}$$

is greater than zero, where $\lambda=t^2V(b_c)/b_c^2$, the suffices 1 and 2 being chosen so that M is greater than zero.

To test the significance of the difference between the values of M obtained in two different experiments for two poisons which have been compared with the same control, we refer $\dfrac{M_1-M_2}{\sqrt{s_{M_1}^2+s_{M_2}^2}}$ to the t-table, with the sum of the number of degrees of freedom in s_{M_1} and s_{M_2}.[1]

As an example, we may test whether the potency of the toxicarol precursor in series 2, Table I is significantly different from that of the toxicarol precursor (by potash separation) in series 3, both having been compared directly with rotenone.

$$M_1=-1{\cdot}1863,\quad M_2=-1{\cdot}1137,$$

$$s_{M_1}=\ 0{\cdot}0287,\quad s_{M_2}=\ 0{\cdot}0420.$$

Hence $$t=\frac{M_1-M_2}{\sqrt{s_{M_1}^2+s_{M_2}^2}}=\frac{-0{\cdot}0726}{0{\cdot}0509}=-1{\cdot}43,$$

with 8 degrees of freedom, which is not significant.

As a second example, compare the Sumatra-type resin in series 4 with the *D. elliptica* resin in series 6.

$$M_1=-0{\cdot}800,\quad M_2=-0{\cdot}5653,$$

$$s_{M_1}=\ 0{\cdot}0247,\quad s_{M_2}=\ 0{\cdot}03225.$$

Hence $$t=\frac{M_1-M_2}{\sqrt{s_{M_1}^2+s_{M_2}^2}}=\frac{-0{\cdot}235}{0{\cdot}0406}=-5{\cdot}79,$$

[1] This test must, of course, be used with caution. It would scarcely be relevant to compare two poisons from two experiments carried out under widely differing conditions.

with 10 degrees of freedom. This value is highly significant, the 1% point being 3·17. Thus the potency of the *D. elliptica* resin is significantly greater than that of the Sumatra-type resin.

Where the values of s_{M_1} and s_{M_2} differ so widely that it is clear that one of the pair M_1, M_2 is determined much more accurately than the other, no exact test of significance can at present be given. In this case, which does not arise in any of the present experiments, it is safest to give to the value of t the number of degrees of freedom in the *larger* of s_{M_1} and s_{M_2}, instead of the sum of the number of degrees of freedom in s_{M_1} and s_{M_2}.

SUMMARY

1. An account is given of the preparation and a few of the properties of a compound isolated from the extracts of Sumatra-type derris root.

2. This compound although in the present investigation containing extraneous material, probably sumatrol, yields optically inactive toxicarol in high yield, and is characterized by the switch-over from laevo- to dextro-rotation on the addition of caustic potash in methyl alcohol to its benzene solution, and is mainly responsible for this feature of the Sumatra-type resins under similar treatment. The change-over in rotation was followed by a gradual fall in rotation of a unimolecular type. The compound is laevo-rotatory in benzene and dextro-rotatory in alcohol.

3. The toxicities to *Aphis rumicis* of rotenone, toxicarol precursor, sumatrol, toxicarol and the residual resins from the Sumatra-type and *Derris elliptica* roots have been determined. In our experiments the toxicity in descending order was Rotenone > *D. elliptica* resin > Sumatra-type resin > sumatrol = toxicarol precursor > inactive toxicarol.

4. The statistical method used in analysing the insecticidal data is described in an Appendix.

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NOTE

A FURTHER NOTE ON FUNGUS ASSOCIATION IN THE SIRICIDAE

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An investigation into the relationship existing between fungus and insect in the Siricidae was commenced in 1928 on the suggestion of Dr R. Neil Chrystal.

Buchner (1928) had described and figured the occurrence in *Sirex gigas* of paired glandular structures which occur at the base of the ovipositor. These glands open into the insect's vagina and in them fungus "oidia" were observed by him. These "oidia" in some cases possessed clamp connexions and the fungus was, therefore, placed in the Basidiomycetes. A note on this investigation was published by the author (Cartwright, 1929), *S. cyaneus* being the species mainly under investigation. *S. gigas* was only cursorily examined owing to lack of adequate material. Since this note was published the investigation has been continued as material became available and as time permitted. Material of both *S. cyaneus* and *S. gigas* has been received from several different localities, and a limited amount of material of *Xiphydria prolongata*. It is proposed, when the entomological side of the investigation has been completed, to publish a full account, but a summary of progress is given here in the hope that it may prove of interest to other workers in the same field.

Since the publication of the original note (Cartwright, 1929) the results there recorded in respect of *Sirex cyaneus* have been confirmed. A fungus has been isolated from glands, eggs, oviposition and larval tunnels of both *S. cyaneus* and *S. gigas*. It has not been possible to establish with certainty the identity of the fungus associated with *S. cyaneus*, but it closely resembles that obtained from *S. gigas* and, although there are certain constant differences, these are hardly sufficient to warrant considering it more than a form of the same species. The culture is characterized by the formation as it ages of a greyish lilac colour, this coloured area possibly representing a rudimentary hymenial surface. The colour resembles that of the fruit body of *Peniophora quercina*.

The mycelial characters of the fungus associated with *Sirex cyaneus* are very similar to those found in *Stereum sanguinolentum*, and cystidia with crystalline incrustations are produced, but in greater abundance. In particular, the sweet odour characteristic of *S. sanguinolentum* is pronounced. A few basidiospores were produced in one culture and these came within the range of measurement of those of *S. sanguinolentum*. The rate and type of growth on malt agar is also very similar. On the other hand, a constant difference is apparent between typical *S. sanguinolentum* and all isolations from *Sirex cyaneus*. This is the pinkish cinnamon colour produced in cultures of the latter as compared with the more yellowish tints produced in the former. Another point of difference is that the fungus in the case of *S. cyaneus* appears to develop a definite mycelial growth which invests the egg previous to oviposition, whereas in the case of *S. gigas* the short segments remain in this condition until after

oviposition, but an insufficient number of insects have been so far examined to state whether this difference is a constant one. As there is a fairly wide variation in cultural characteristics between different isolations of *Stereum sanguinolentum*, it is thought probable that the fungus associated with *Sirex cyaneus* is a form of *Stereum sanguinolentum*, although, until characteristic fruit bodies are obtained, it is not possible to confirm this opinion.

The fungus isolated from *Sirex gigas* has been identified by means of comparison with standard cultures as *Stereum sanguinolentum*. Small samples of larch were inoculated with a culture isolated from a gland of *Sirex gigas*, and fruit bodies of *Stereum sanguinolentum* were produced on the wood, thus confirming the identification. Further corroborative evidence was obtained by the formation of fruit bodies of this fungus appearing on wood samples from which *Sirex gigas* had emerged after these had been kept moistened in a potato dish in the laboratory.

Under the moisture conditions occurring in wood in which live larvae are present no advanced stage of rot has been observed although, in a specimen of larch wood taken from a felled log in which old *Sirex* attack was evident, the decay had progressed to a considerable extent. In one instance a pupa of *S. gigas* was sent to me by Dr Chrystal, which was dead and surrounded by a weft of mycelium. The fungus proved to be *Stereum sanguinolentum*. In this case the moisture conditions had apparently become favourable for the rapid development of the fungus, with fatal results to the *Sirex*. It appears that conditions of moisture in the wood favourable to *Sirex* are those that are just sufficient to support a slow development of the fungus.

In both the case of *S. cyaneus* and *S. gigas* there is very strong evidence for stating that a definite species of fungus is associated with each species of insect in so far as this country is concerned, and further evidence to the same effect has been obtained by Clark (1933) working in New Zealand with *S. noctilio*.

Females of both *S. cyaneus* and *S. gigas* have been observed ovipositing in wood free from all traces of fungus; the tunnels have been opened up and the eggs removed immediately after oviposition, and also at varying times after oviposition. Sections cut across these oviposition tunnels show all stages of development of the fungus from the egg, so that no doubt remains but that the fungus is introduced into the wood during oviposition.

Clark (1933) in New Zealand has carried the work a stage further, claiming to have found fungus in the larva apart from that which is obviously present in the digestive system owing to the larva feeding on wood containing fungus. In my preliminary examination, fungus was observed in a partially digested condition in the larval gut. Sections across late stage pupae also showed fungus to be present in glands at the base of the ovipositor in the case of female pupae.

Work has now commenced following up the development of the special fungus-carrying glands with a view to finding at what stage in the life history of the insect the fungus becomes segregated in these structures; this is being investigated by my entomological colleagues.

Xiphydria prolongata.

A small quantity of willow containing this insect has also been examined. Up to the present the investigation has been confined to the isolation of the fungus from the adult female and from the egg and larval tunnels. From the original material

received, *Stereum purpureum* was isolated from old larval tunnels, but from material in which larvae were still active, and from which adults emerged later, isolations both from larval tunnels and from glands from the adult female have given a culture resembling closely in macroscopic appearance that of *Daldinia concentrica*, but differing from it microscopically in certain respects.

The fungus belongs almost certainly to the Pyrenomycetes and is one which can cause some decay of the wood.

SUMMARY

1. Earlier work on the association of *Sirex cyaneus* with a Basidiomycete fungus has been confirmed. The culture of the fungus closely resembles, but is not identical with that of *Stereum sanguinolentum*; it is therefore thought that it is a form of *S. sanguinolentum*.

2. The fungus isolated from *Sirex gigas* has been identified as *Stereum sanguinolentum*.

3. At the moisture content favourable for the development of the larvae, only slow development of the fungus can take place. Under more moist conditions vigorous fungus growth occurs, with apparently fatal results to the larvae.

4. The fungus is introduced into the wood during oviposition in the case of both *Sirex cyaneus* and *S. gigas*.

5. Fungus was found to be present in glands at the base of the ovipositor in late stage female pupae.

6. A fungus, the culture of which resembles in appearance that of *Daldinia concentrica*, has been isolated from larval tunnels and from glands in adult females of *Xiphydia prolongata*.

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(*Received* 1 *December* 1937)

REVIEWS

Statistical Methods. By G. W. SNEDECOR. Pp. xiii+341. Ames, Iowa Collegiate Press Inc. 1937. $3.75.

No statistician whose occupation brings him into contact with biological research workers can be ignorant of the urgent demand to-day for a book which will explain statistical methods clearly and authoritatively. It is such a book that Prof. Snedecor has set out to write. In this respect the book perhaps calls for two reviewers, for while the professional statistician may judge with confidence the correctness and utility of the methods explained, he is naturally somewhat handicapped in estimating the appeal which the book will have to a biological research worker with no previous knowledge of statistics.

The author of an introductory text-book must choose whether to present the subjects discussed in a logical sequence or in order of increasing difficulty. The first alternative makes the book more difficult to read, but may result in a more complete and satisfying picture of the whole subject, once the difficulties have been mastered. Snedecor's experience in teaching statistics has led him to choose the latter method. This method certainly makes every individual chapter in the book easy to follow, but its success in presenting the whole subject depends on the ability of the reader to collate discussions widely separated in print. In this task he is, however, given every assistance by the author.

The book opens with a discussion of the test of significance of the departure of an observed percentage from a hypothetical value (the χ^2 test for a single degree of freedom). Then follows an account of a replicated experiment with two treatments only, in which the meaning and calculation of the standard error are explained. Chapters III and IV are devoted to sampling from a normal population, and introduce the reader to the t-test and to the idea of fiducial probability. Chapter V gives some useful short cuts in computation. The succeeding chapters discuss in turn linear regression, correlation, large sample theory (including the use of grouping and the tests of departure from normality) and we return in Chapter IX to the χ^2 test, with more than one degree of freedom. The chapter on correlation is excellent; it stresses the limitations of the correlation coefficient both in its utility and in its ability to measure association.

Thus far, the succession of subjects is logically somewhat haphazard. The remainder of the book, with the exception of the last chapter, concerns the analysis of variance, and includes the analysis of co-variance, multiple regression, the fitting of curved regression lines and the isolation of single degrees of freedom. The final chapter discusses briefly the binomial and Poisson series.

Prof. Snedecor's style is deliberately conversational, and while the purist may deplore the large number of colloquialisms, the friendly atmosphere which is created is a great help in studying a subject which is often approached by the student with a mixture of awe and hostility. Examples to be worked by the reader are numerous in all parts of the work. In some cases, working of the examples is not merely helpful but essential to obtain a correct understanding of the discussion; e.g. on p. 237 the treatment given in the text is superficial and is a bad precedent for the reader to follow; this is, however, put right in example 12.10, where the general conclusion reached in the text is corrected.

The methods developed are in the main sound and practical. A few points occurred to the reviewer which might mislead the reader. On p. 178, the statement occurs: "The F-test is valid whether the variance is homogeneous or not." Presumably the author means the F-test considered as a general test of differences between samples; however the F-test is always used in practice to discriminate

between *means* and as such is not a correct test if the variances differ widely. On p. 196 a large sample test of the differences between a set of variances is used on a set of variances with only three degrees of freedom each, to which it is certainly not applicable, though the author warns the reader elsewhere of the limitations of large sample methods. The treatment of multiple regression might be more simple and self-contained if the use of the multiple correlation coefficient were omitted. The book ends on an unnecessarily defeatist note, the last sentences reading: "If large samples of enumeration data do not follow either Binomial or Poisson distributions, there is no alternative but the disagreeable one of treating them as normal. Conclusions, if any, should be tentative." Thanks to the use of transformations, this statement is fortunately untrue, as a recent paper in this *Journal* exemplifies (C. B. Williams (1936), **24**, 404). Indeed, the neglect of transformations, in particular the square root, inverse sine and log transformations, is a serious limitation to the use of this book.

Prof. Snedocor is to be congratulated on producing a book which will be of great assistance to biological workers.

W. G. COCHRAN.

Report of the Fourth International Grassland Congress. Edited by R. O. WHYTE. Pp. xxxiv+486. Obtainable from the Joint Secretaries, IVth International Grassland Congress, Aberystwyth. 1937. 40*s*.

This *Report* contains lists of the officers of the Congress, a table of contents, full texts of the Presidential Address, plenary papers, sectional papers, and discussions, the names and addresses of persons attending the Congress, and indexes of authors of papers and contributors to discussions.

A Grassland Congress held within the British Empire could have but one President, R. G. Stapledon, a man of vision and courage whose leadership is everywhere recognized. But in an opening address to a large Congress, any President is almost compelled to range widely and superficially and so, although what Stapledon said is well said and well worth saying, it could hardly be expected to contain anything new.

The authors of the thirteen plenary papers represent nine countries; mostly deal with somewhat wide topics of special importance in their particular countries; and give a good idea of present viewpoints. Grass drying, which is much to the fore in Great Britain, is discussed by Woodman of Cambridge. Vezzani and Carbone of Torino deal with alpine grazing of cattle, a primary issue in Northern Italy. More than 70% of the occupied land of Australia south of the tropic of Capricorn is used for wool growing and Marston of Adelaide, therefore, discusses the nutritive value of pastures for wool production. American plant breeders are perfecting techniques of grass breeding, and clarifying objectives so as properly to relate plant breeding to other grassland improvement activities. Cardon of Washington deals, therefore, with plant breeding in relation to pasture improvement. Sweden is the home of the ecotype concept, and Sylvén of Svalöf considers the importance of ecotype formation for the breeding of herbage plants. In Sweden, grasslands occupy nearly one-half of the total agricultural area and, as grassland management is in a transition state, Osvald of Uppsala discusses achievements and aims in modern Swedish grassland management. At Aberystwyth one of the main themes has been strain building in the herbage grasses and this subject is dealt with by Jenkin. Pedigree grasses need to be multiplied, distributed and grown, and the part played by seedsman and farmer is considered by Miln of Warrington. In New Zealand, about 14 million acres of forest have been felled and sown to grass, and about 2 million acres of fern and scrub land: awaiting development are still about 2 million acres of fern and scrub land and some 4 million acres of standing forest. This problem of the conversion of rain forest to grassland is discussed by Bruce Levy of Palmerston North. It has long been known that non-legumes derive benefit when grown in association with legumes, but the phenomenon has presented obscurities. During the last decade it has been studied by Virtanen of Helsinki who puts forward an explanation. In Canada the few species of grasses and legumes that are grown extensively are highly adapted to the rather exacting climatic

conditions, and Kirk of Ottawa discusses their evaluation for pasture. In world agriculture soil erosion has become an imperative issue, and a recent survey has shown that, in the United States, about 100 million acres are so seriously eroded as to be practically worthless for continued cropping, while a further 100 million acres are rapidly becoming worthless. The outstanding value of pastures in soil erosion control is now recognized and is discussed by Enlow of Washington. In Germany, grassland research is well advanced, and enables Klapp of Bonn to consider the principles governing the value of herbage plants for hay and pasture use. These plenary papers contain nothing strikingly new, but they are interesting and serve to emphasize some of the main general issues confronting world agriculture to-day.

The Congress was organized in six sections as follows: (1) Grassland ecology, including range management; (2) Seeds mixtures, legumes for use in poor pastures; (3) Plant breeding, genetics and seed production; (4) Manures and fertilizers, soil aspects of grassland; (5) Nutritive value of pastures, fodder conservation; (6) Pastures. Management, yields and economics. To a large extent the sectional papers deal with the same general problems as the plenary papers but help to fill in the details and, as the contributors represent eighteen countries, the papers embody an unusual width of knowledge and experience. It is not possible to review the fifty-five sectional papers, or even to mention individual contributions. On the whole the papers are of high quality and many of them are exceedingly interesting. Grassland diseases are not considered, and only one paper is devoted to grassland pests.

During an evening spent in browsing these pages two general ideas gradually formed in my mind. The first was that in spite of the enormous and rather diffuse range of grassland problems, with their apparent complexities, and their manifold impacts upon all sorts of correlative agricultural, economic and social issues, the general principles underlying the utilization and development of grasslands are few in number and comparatively simple. The second was the fundamental importance of grasslands in social economy and, arising out of this, the certainty that the improvements which could be effected in grasslands within a relatively short period would so materially influence the economic and social structure of many countries, that the changes would amount to a sizable revolutionary movement. Friend Stapledon will have much to answer for.

The *Report* is a good cross-section of a vital subject and a valuable storehouse of data and viewpoints. The Editor has had no light task, and must earn praise for the way in which the volume has been produced and for its rapid issue after the Congress. The *Report* would have been graced by a good portrait of the rather elusive Congress President.

WILLIAM B. BRIERLEY.

Studies on Wheat Grown under Constant Conditions: a Monograph on Growth. By H. L. VAN DE SANDE-BAKHUYZEN. Pp. xvi + 400. Food Research Institute, Stanford University, California. 1937. $4.00.

In biology there are, as yet, no exact values which can be used as the hydrogen atom is used as a basic datum in chemistry, or the 100% in mathematics. If there were a standard plant with which any other plant could be compared, botany would immediately become a much more exact science. The rapidly accumulating knowledge of the nutritional requirements of plants and the development of the technique of growing plants in chambers in which environmental conditions are strictly controlled, give promise that it might be possible to obtain such an intensive knowledge of a single plant that that plant could serve as a datum line in botany. A selected pure line of wheat grown under constant conditions might well be accepted as such a standard plant, and the importance of the present work lies in the fact that it may be regarded as a first approach to such an ideal. The book has grown out of crop forecasting studies and the fact that some of the correlations observed could not be explained on the basis of existing knowledge regarding the physiology of growth.

It was decided, therefore, to make a study of the wheat plant organ by organ under an unvarying environment, and to use this knowledge as a standard with which to compare the growth with single variables modified.

Chapter I is an admirable general introduction to the physiology and development of the wheat plant. Seven chapters follow dealing with the growth curve in annual plants, six with materials and methods, three with general growth features, two with dry weight and moisture content of the different organs, and four with dry weight and moisture of the "standard plant". The next two chapters discuss and summarize these data. There follow four chapters dealing with the nitrogen and carbon of the organs, six with nitrogen metabolism in relation to growth and development, and a final chapter in which these data are discussed and summarized. The book concludes with a useful bibliography citing references up to 1935 when the manuscript was completed in almost its present form, and an index. Much of the first nineteen chapters is reprinted with modifications from *Physiological Reviews* and *Plant Physiology*; and Elizabeth P. Griffing and Carl L. Alsberg have contributed to the sections dealing with the carbon and nitrogen content of the wheat plant.

Quite apart from the intrinsic value of the data, the work is of importance in that it lies within a comparatively untouched field. There have, of course, been numerous researches on the pathological relationships of plants growing under controlled conditions, and on various aspects of the physiology of detached organs maintained under controlled conditions, but there has been astonishingly little investigation of the general physiology of complete plants growing in a determined environment. In the present work the factors controlled were temperature, light, humidity of the air, moisture and salt content of the substratum (sand or solution). The present work is to be regarded rather as the initiation of a programme for future work than as the completion or solution of a problem. Even so, however, it is of the greatest interest and its continuance may easily lead to a new valuation of some of the more fundamental concepts and problems of plant physiology. By itself the attempt to work out the idea of a "Standard Plant" well justifies publication.

WILLIAM B. BRIERLEY.

Phytohormones. By F. W. WENT and K. V. THIMANN. Pp. xi+294. New York: The Macmillan Co. 1937. 17*s*.

The birth of the growth hormone concept *per se* may be dated 1919 with the publication of Paál's second paper on the phototropic stimulus. The suggestion of the existence of organ-forming and correlative substances goes back to the writings of Duhamel du Monceau in 1758 and was re-stated in different terms by Sachs (1880–93) and by Beijerinck in 1897, while Darwin conceived the idea of phototropic and geotropic curvatures being due to "some influence" which "is transmitted from the upper to the lower part, causing the latter to bend". Rothert, Fitting, and Boysen-Jensen laid more of the experimental foundation, but it was left to Paál to show the relation of the phototropic response to a material substance which is active in promoting *normal* growth.

The formulation of a clear-cut concept gave a great impetus to experimental study and the final material proof was provided by the skilful chemical work of Kögl, Haagen Smit, and Erxleben in isolating and analysing the auxins and in showing that these substances produced reactions identical with those previously obtained with root and shoot tips.

The establishment of the relation of these growth-promoting substances to many well-known correlations in organ-development, such as bud inhibition and root initiation, has led not only to the elucidation of many previously obscure problems but to the development of an entirely new outlook on the physiology of growth and behaviour in plants. It is, possibly, not too much to suggest that the hormone concept may rank in the history of plant physiology with the cell-theory in that of morphology or the binomial principle in systematics. As the authors themselves say, "we already

see the auxins and their properties as a continuous thread connecting most of the developmental and growth processes in the plant".

Indicative of the vast amount of work which the concept has stimulated is the fact that although the theory is not yet out of its teens two important books, both containing solidly condensed experimental results, should already have appeared, the present volume and that of Boysen-Jensen (*Die Wuchsstofftheorie*). Of the two, there can be no question that the book under review is far the more convincing, comprehensive and stimulating. It is only to be expected that two investigators who have themselves contributed a large part of the soundest fundamental work but who have, at the same time, an imaginative and synthetic outlook should occasionally tend to explain too much from too little. We cannot, as yet, understand certain of the phenomena associated with the action of growth-substances, nevertheless, an hypothesis is suggested in almost every case. It is, however, just in this respect that the book is so stimulating.

The main point on which issue will be joined by many physiologists is the tendency to discount the nutritional outlook and to explain, not only to relate, all growth phenomena in terms of auxin production and distribution. For example (p. 231), "the root system probably forms a factor or factors necessary for shoot growth and *hence* the increased root system increases the shoot" (italics are ours), or again, and even more categorically, (p. 232) "quantitative relations between different parts of the plant are expressions of the quantitative relation between auxin and its growth effects. This generalization can now supersede the older view that such growth relations are determined by the amounts of food material present". Truly a revolution in outlook.

Those who seek in this book for a lengthy and detailed treatment of the practical applications of growth substances in horticulture, especially in the rooting of cuttings, will be disappointed. The latter subject receives a bare two pages of text and one illustration, and the very considerable number of papers on it are scarcely noted, four references only being quoted. It is to be hoped that in future editions this section will be expanded, even though the book is primarily a discussion of the fundamental principles of the subject.

There can be no doubt that the work represents an immensely important contribution to plant physiology and it is to be hoped that it will be read and deeply considered not only by the believers but, also, by the diehard minority who still dismiss the hormone concept as a will-o'-the-wisp.

The book is divided into fourteen chapters. After a brief review of the development of the concept, a condensed but adequate account is given of the technique of auxin determinations, followed by chapters on the formation, distribution and chemistry of the auxins. Theories of the mechanism of action are discussed at length, the remaining chapters being concerned with the tropistic and correlative activities of the substances, and general conclusions and future outlook. The book concludes with a bibliography of 569 references, further evidence of the intensive study of the most productive physiological conception of this century.

R. H. STOUGHTON.

A Review of the Literature on Stock-Scion Incompatibility in Fruit Trees, with Particular Reference to Pome and Stone Fruits. By G. K. ARGLES. Pp. 115. Tech. Communication No. 9. Imperial Bureau of Fruit Production, East Malling, Kent. 1937. 5*s*.

A good practical review of this problem under the general headings: Discussion on the manifestations or symptoms of incompatibility; Possible causes of incompatibility; Stock-scion incompatibility in relation to individual species and varieties of deciduous fruit trees; Conclusions and suggestions. The above occupies the first half of the bulletin and is followed by two pages containing interesting suggestions for further research under the headings: Factors governing the success or failure of

buds and grafts to form a union; The growth of trees on different rootstocks; Tests to determine the mechanical strength of unions; The anatomical structure of unions; Translocation in grafted trees; Physiological and biochemical studies. There is a bibliography of 194 references. A 46-page appendix contains six lists of rootstocks which have been used, either experimentally or commercially, for varieties of pears, plums, peaches, apricots, almonds, and cherries. Apples are omitted owing to the relatively few cases of incompatibility which have been reported. The lists are arranged in parallel column under species, variety and method of propagation of the stock, species and variety of the scion, behaviour of the union, where the observations were made, and reference citation.

WILLIAM B. BRIERLEY.

Forest Bibliography to 31*st December*, 1933. Compiled and published by The Department of Forestry, University of Oxford. Part 1, pp. xviii + 1–78. 1936. 5*s*. Part 2, pp. 79–199. 1937. 12*s*. 6*d*.

A systematic referencing of current forest literature was begun at the Oxford School of Forestry in 1920 and, after 1924, continued jointly by the School of Forestry and the Imperial Forestry Institute, the bulk of the work being done by Mr P. S. Spokes. At first publication was not intended, the object being to keep staff and students in touch with the latest literature bearing on forestry, but the index was found so useful that, in response to requests from various sources, it was decided to publish the work. The Bibliography comprises literature published to the end of 1933 and contained in the library of the Department. English publications are covered fairly completely and a considerable amount of French and German literature is also included, but few publications in other languages are represented unless they contain English, French or German summaries.

Part 1 commences with 14 pages of the names of journals, and of series of bulletins, reports, etc., with their abbreviated titles. Many of these are of very special character and so omitted from the Oxford *World List of Scientific Periodicals*, but it is a great pity that the standard abbreviations of such journals as are included in this work could not have been adopted. Four pages are then devoted to a Subject Classification which forms the basis of the bibliographical arrangement. The major headings are: A, General Forestry; B, Silviculture; C, Forest Protection; D, Forest Utilization; E, Forest Mensuration; F, Forest Valuation and Finance; G, Forest Management; H, Forest Policy and Economics; I, Meteorology; J, Geology and Soil; K, Education and Research; L, Terminology and Classification of Information; M, Engineering and Surveying; N, Botany; O, Invertebrate Zoology; P, Bibliography. Each section is further subdivided under headings. The two parts of the Bibliography now issued cover the literature to the end of Silviculture (i.e. A and B). The citations are grouped under two heads: (1) periodicals (2) other publications; books are excluded. The detailed arrangement on the pages is by parallel columns under date: year, abbreviated title of journal, volume, page, title of paper, and name of author.

When it is realized that about 750 journals, etc., are dealt with, some dating as far back as the early nineteenth century, the magnitude of the task may be appreciated. The indexing has been done with meticulous care and, when completed, the Bibliography will be an indispensable work of reference.

WILLIAM B. BRIERLEY.

Applied Mycology and Bacteriology. By L. D. Galloway and R. Burgess. Pp. ix + 186. London: Leonard Hill, Ltd. 1937. 10*s*.

Applied microbiology is a large and rapidly developing field of research, and whilst numerous volumes dealing with special aspects and problems are available, there has been no up-to-date book of convenient size in which the whole field was surveyed.

The present book fills the gap admirably since both authors have had wide practical experience of the subject.

The book is divided into two parts. Part 1, with eight chapters, is in a sense introductory, and deals with the generalities and classification of the fungi and bacteria, methods for their study, their metabolism, and their control. The material is well chosen and includes the essentials, and the authors have condensed readably into small compass a mass of data and sound practical knowledge. Part 2, with six chapters, is devoted to the applications of microbiology in the food, fermentation and textile industries, medicine and hygiene, agriculture, and various miscellaneous problems. This portion occupies only 73 pages, but the scope of the treatment and the amount of matter contained are astonishing. The authors state that "Medical microbiology has been barely touched on, and admittedly deserves a larger space than it receives here." On the other hand this is one of the specialized applications on which there is a superabundance of readily available books, and the authors have been wise in restricting their attention to the more general hygienic issues. The final miscellaneous chapter deals briefly with timber decay, wood pulp and paper, rubber, leather, paints, moulds and arsenic compounds, tea, coffee, cocoa, indigo, tobacco, vegetable oils, the use of micro-organisms for chemical analysis, and a final interesting section on future developments in economic microbiology. Key references are cited at the end of each chapter, and these are commendably up to date. The book closes with an adequate index.

This book is the best introductory treatment of the subject I know. It would form an excellent basis for a lecture course on applied microbiology, or first-class reading for any student of botany who might wish to adventure beyond the academic pale of his mycological studies.

WILLIAM B. BRIERLEY.

A List of Missouri Fungi. By W. E. MANEVAL. Pp. 150. University of Missouri Studies. 1937. xii (3). $1.25.

A list of plant pathogens and wood-destroying fungi including, also, a small number of saprophytes, bacteria, nematodes, and diseases caused by viruses and environmental conditions. The organisms are arranged alphabetically by genus and species, with host plants listed under each species and, in all, there are some 1330 organisms and diseases on about 700 different hosts. There is a host index with the fungi, etc., arranged alphabetically under each host species. The volume opens with a review of the literature dealing with Missouri fungi and closes with a bibliography of 526 references. The work seems to have been well done and there are commendably few misprints.

WILLIAM B. BRIERLEY.

Technique of Grass Seed Production at the Welsh Plant Breeding Station. By GWILYM EVANS. Herbage Publication Series, *Bull.* No. 22. Pp. 36, 24 figures. Aberystwyth: Imperial Bureau of Plant Genetics. 1937. 5*s*.

The grasses bred at the Station are *Lolium perenne, L. italicum, Dactylis glomerata, Phleum pratense, Festuca pratensis, F. rubra, F. ovina, F. arundinacea, Alopecurus pratensis, Poa pratensis, Holcus lanatus, Anthoxanthum odoratum, Phalaris arundinacea* and *P. tuberosa.* The first stage multiplication from a limited number of finally selected plants is carried out at or near the Station; the second stage multiplication on a limited field scale, and the third stage with several fields for each strain, by growers in favourable districts. The three stages are described, the greater part of the bulletin concerning stage three, where details are given regarding selection of centres, field management, harvesting, treatment of harvested crops, successive seed crops,

and crops to follow grass seed crops. There are notes on the production of seed for general distribution and on the bred strains of grasses and clovers released by the Station.

WILLIAM B. BRIERLEY.

Production of Grass Seed. Herbage Publication Series. *Bull.* No. 19. Pp. 46. Aberystwyth: Imperial Bureau of Plant Genetics. 1937. 5*s*.

An international exchange of opinions and experiences on the technique of producing seed of *Alopecurus pratensis*, *Bromus inermis*, *Cynosurus cristatus*, *Dactylis glomerata*, *Phleum pratense*, *Poa pratensis*, and various species of *Agropyron*, *Agrostis*, *Festuca* and *Lolium*. Contributors represent Scotland, Northern Ireland, Canada, New Zealand, Germany, Sweden, and U.S.A.

WILLIAM B. BRIERLEY.

Collection of Native Grass Seed in the Great Plains, U.S.A. By F. J. CRIDER and M. M. HOOVER. Herbage Publication Series, *Bull.* No. 24. Pp. 8, 12 figures. Aberystwyth: Imperial Bureau of Plant Genetics. 1937. 2*s*.

The grasses included are *Agropyron smithii*, *Andropogon scoparius* and *A. furcatus*, *Buchloë dactyloides*, *Sporobolus airoides*, and *Bouteloua gracilis*.

WILLIAM B. BRIERLEY.

Production of Legume Seed. Herbage Publication Series, *Bull.* No. 23. Pp. 48. Aberystwyth: Imperial Bureau of Plant Genetics. 1937. 5*s*.

An international exchange of opinions and experiences on the technique of producing seed of *Anthyllis vulneraria*, and various species of *Glycine*, *Lespedeza*, *Lupinus*, *Medicago*, *Melilotus*, *Trifolium*, and *Vicia*. Contributors represent Australia, Canada, Great Britain, India, Czechoslovakia, France, Germany, Hungary, Sweden, and U.S.A.

WILLIAM B. BRIERLEY.

Weeds, Weeds, Weeds. By Sir CHARLES VERNON BOYS. Pp. 69. London: Wightman and Co., Ltd. 1937. 1*s*.

A pleasantly written and popular little book describing the author's personal experiences in the control of weeds by various chemicals, and containing much sound advice. The author shows unusual abandon in his use of small and capital letters in the spelling of scientific names.

WILLIAM B. BRIERLEY.

Recent Advances in Entomology. By A. D. IMMS. London: J. and A. Churchill, Ltd. 2nd edition. 1937. 15*s*.

Dr Imms has once more increased our indebtedness to him by the publication of the second edition of *Recent Advances in Entomology*. The subjects dealt with are the same as in the first edition (noticed in this *Journal* in 1931, vol. **18**, p. 262) but the book has been enlarged by some 55 pages and many of the chapters have been largely re-written. The field of work open to entomologists is now so vast that none

of us can hope to keep abreast of all advances in knowledge. Dr Imms gives us, as we have learned from his *Text-book* and his first edition of *Recent Advances*, the results of wide and critical reading in what we may call the grammar of entomology on which he is the acknowledged leader in this country.

The first eight chapters of his book are devoted to this and in them his active and critical interest is manifest. In the later chapters on ecology and its applications and on parasitism, too, he sums up skilfully the important new contributions and their implications.

When he deals with the control of insect pests and weeds by the use of parasites, however, his critical lance seems to lie in rest and his account of "biological control" is virtually a repetition of the published views of the authors cited.

Dr Imms follows the majority of workers on the control of insect pests by parasites in using the term "biological control" to mean solely control by parasites. For example, he deals with Parnell's production of the jassid resistant cotton U4 in his sub-chapter on "resistant varieties" although surely Parnell's work is one of the finest examples of the biological control of an insect pest we have had for many years. In the writer's view, this restriction of the term biological control is much to be regretted, both on theoretical and practical grounds. Dr Imms, however, apparently agrees with the restriction for he states the "entomological viewpoint" as follows:

"Among the natural agencies exercising a controlling influence upon insect life the complex of meteorological factors constituting climate is ever present. Simultaneously another complex of a biological nature is exerting its influence, and represents the sum total of the activities of bacterial, fungal and other diseases, of insectivorous birds and mammals and of parasites and predators. The restraint exercised by these several biological agencies is known as biological control, and in so far as insect life is concerned, the influence of parasites and predators alone is of immense significance."

It is striking that, in enumerating the agencies which govern fluctuations in insect numbers, Dr Imms completely omits the plants which afford sustenance for many insects, especially nearly all insects injurious to agriculture and forestry. It is the more remarkable because in the sentence which immediately follows the quotation above, he says: "Plant life is likewise affected by a complex of biological agencies including fungal, bacterial, and other diseases, together with the depredations of those *insects directly dependent upon vegetation for their sustenance*."[1]

Why is it that in nearly all discussions on the fluctuations of insect populations conducted by those interested in the parasitic hymenoptera and diptera the sustenance factor is ignored? One would almost think that Malthus, who by his axiom that all animal life tends to increase to the maximum of its subsistence, inspired both Darwin and Wallace to consider the origin of species by natural selection, is completely forgotten. In spite of his last statement quoted, Dr Imms proceeds immediately to discuss "Biological Control of Insect Pests" and once more reverts to a discussion in which the plant is forgotten and insect pest populations are isolated from their environment except in so far as parasites are concerned. In fact a few lines later Dr Imms refers to Dr Nicholson's papers on the principles underlying control by parasites and particularly emphasizes Nicholson's reference to the "fact that if there is a balance in the population of a particular insect some of the factors causing mortality must be dependent upon past density and destroy a greater proportion of the individuals of a specific pest when the density of the latter is high than when it is low". He adds that "in the case of parasites and predators it is assumed that they search for their hosts at random, and it is shown that the mortality which they cause will be dependent upon host-density. Since climate and *other factors* operate irrespective of such density, parasites and predators are regarded as the main factors bringing about balance".

Like Dr Nicholson, Dr Imms is prepared to discuss the parasite and its host, the pest insect, and ignore the fact that it also has a host and that the density of the pest host may be dependent on the plant host and that that plant host cannot be

[1] My italics.

ignored. That it should be ignored is the more surprising if we remember that it is the plant host and its protection that is the reason for the whole discussion.

It is entirely desirable that theories of insect populations should be formulated and discussed, but theories must start with premises, and their value will depend almost entirely on whether the premises are sound or faulty. That authors of papers published in scientific journals should sometimes let their zeal for theorizing outrun their common sense and discretion is pardonable; the danger is lest students of Dr Imms' book, still finding their way in entomology, should be misled by the discussion just quoted. Already, at least two schools of thought concerning the factors governing fluctuations in insect populations have appeared among us, the one represented by Prof. Buxton and Dr Uvarov, for example, emphasizing the importance of the climatic factor, the other represented by the late Dr Tillyard and Dr W. R. Thompson, for example, emphasizing the importance of the parasite factor. Both schools are partly right but neither is completely right and the real need is to suspend judgement, to discriminate and above all to acquire more knowledge without bias.

One sympathises with Dr Imms if, in a later edition, he seeks to give a balanced account of insect population problems, because so much is written on parasitism and on climatic factors and so little on the food supply or subsistence factor, but it is much to be hoped that he will make the attempt, for his *Recent Advances*, in other respects, sets a standard of critical compilation which is admired the world over.

J. W. MUNRO.

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1937

THE officers and Council of the Association were as follows:

President: J. HENDERSON SMITH, M.B., Ch.B.

Vice-Presidents: S. P. WILTSHIRE, M.A., D.Sc.
C. T. GIMINGHAM, B.Sc., F.I.C.

Hon. Treasurer: J. HENDERSON SMITH, M.B., Ch.B.

Hon. Secretaries: General and Botanical, W. P. K. FINDLAY, M.Sc.
Zoological, G. FOX-WILSON.

Hon. Editors of *Annals of Applied Biology*:
General and Botanical, Prof. W. B. BRIERLEY, D.Sc.
Zoological, C. T. GIMINGHAM, B.Sc., F.I.C.

Council: H. F. BARNES, M.A., Ph.D., A. S. BUCKHURST, A.R.C.S., W. J. DOWSON, M.A., D.Sc., T. GOODEY, D.Sc., H. MARTIN, D.Sc., H. W. MILES, D.Sc., Ph.D., H. C. F. NEWTON, B.Sc., G. SAMUEL, M.Sc., E. R. SPEYER, M.A., H. G. THORNTON, D.Sc., S. P. WILTSHIRE, M.A., D.Sc., H. WORMALD, D.Sc.

Mr H. C. F. Newton was co-opted by the Council to fill the vacancy resulting from the death of Dr Maldwyn-Davies.

The Association has met on six occasions during the year, including one Field Meeting and one afternoon visit. The Annual Summer Meeting was held on 4 June at the Experimental and Research Station, Cheshunt, by kind permission of the Director, Dr Bewley. The afternoon visit on 11 December was to the gardens of the Zoological Society of London. To both these Institutions the Association is indebted for their hospitality.

At ordinary meetings the attendance as recorded in the signature book was, on the average, 33 members and 17 visitors.

Eighteen Ordinary Members were elected during the year and ten Members resigned. The Council has to record with regret the death of four Members, Prof. Borthwick, Dr Maldwyn-Davies, Miss Hoggan, and of Prof. Paine who acted as General and Botanical Secretary of the Association from 1923 to 1926.

The Association now numbers 314 Members including 12 Honorary Members: of the Ordinary Members as far as is known 252 are resident in the British Isles and 50 in the Empire or Foreign Countries.

The following papers and discussions were brought before the Association during 1937:

12 *Feb.* *Recent Work on the Death-Watch Beetle,* Xestobium rufovillosum.
R. C. FISHER, B.Sc., Ph.D.: "Life history studies."
F. R. CANN, D.I.C.: "Occurrence in buildings and methods of control."

Some Recent Developments in Fumigation.
A. B. P. PAGE, Ph.D.: "Applications and distributions of fumigants."
O. F. LUBATTI, Ph.D.: "Detection and determination of fumigants."

The Use of Liquid Insecticides against Warehouse Pests. C. POTTER, Ph.D.

12 *Mar.* *The Independence and Interdependence of Branches.* W. A. Roach, D.Sc.
Notes on Plant Disease Control in the U.S.A. and Canada. R. W. Marsh, M.A.

8 *Oct.* *The Wireworm Problem, with special reference to the North West of England.* H. W. Miles, D.Sc.
The Rook in the Rural Economy of the Midlands. A. Roebuck, N.D.A.
The Food Habits of the Little Owl. Miss A. Hibbert-Ware, M.B.O.U.

5 *Nov.* *The Marssonina Disease of Lettuce.* Miss G. Stevenson.
The Significance of the Climax for British Forestry. A. S. Watt, B.A., Ph.D.

During the past year the Association has again enjoyed the privilege of holding its meetings in the Botanical Department of the Imperial College of Science and Technology and in the Metallurgical Lecture Theatre of the Royal School of Mines, and the Council takes this opportunity of recording its grateful thanks on behalf of the Association to the College Authorities for their valued hospitality.

The Hon. Editors of the *Annals of Applied Biology* report that in 1937 the volume comprised pp. xiv + 940 and 51 plates, as against pp. xiv + 921 and 39 plates for 1936. Including papers published as "Proceedings", vol. xxiv contained 67 papers and 44 reviews, as against 64 papers and 32 reviews for vol. xxiii. Of the papers in the 1937 volume, 41 were by members of the Association and 26 by non-members. The papers may be roughly classified as follows: plant physiology and non-parasitic diseases 13, mycology and fungus diseases 10, bacterial diseases 2, virus diseases 7, applied entomology 17, plant protection 11, microbiology of soil, etc. 4, general 2, apparatus 1.

The several parts of vol. xxiv of the *Annals of Applied Biology* were published on the following dates:

Part 1. 4 March.
Part 2. 26 May.
Part 3. 17 August.
Part 4. 17 November.

W. P. K. FINDLAY
G. FOX-WILSON } *Hon. Secretaries.*

REPORT OF THE HON. TREASURER FOR THE YEAR ENDING 31 DECEMBER 1937

During the year ending 31 December 1937 subscriptions and entrance fees, including arrears paid in, amounted to £337. 7*s*. 0*d*., almost exactly the same amount as last year. Income from the sale of the current volume of the *Annals of Applied Biology* and from reprints amounted to £809. 5*s*. 0*d*., an increase over last year of £108, mostly attributable to increased reprint sales. The size of the *Annals* was again increased as compared with last year and amounted to 940 pages as against 728 pages in 1934, and the cost of producing it rose to £1330, an increase of £120 over last year's figures.

On the whole year there has been an excess of expenditure over income of £100. 6*s*. 2*d*. as against £101 last year, the increased cost of the *Annals* being compensated by a larger sale of reprints and the greater value of the stock.

The financial position of the Association is sound, with an excess of assets over liabilities of £852; but if we are to maintain the *Annals* at its present size it is essential that we increase our membership. At present the number of new members just compensates those lost through death and resignation and it is very desirable that every opportunity should be taken of obtaining additional members.

J. HENDERSON SMITH,
Hon. Treasurer.

THE ASSOCIATION OF APPLIED BIOLOGISTS

ANNALS OF APPLIED BIOLOGY INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1937

Dr. — EXPENDITURE

	£ s. d.	£ s. d.
To Estimated Value of Stock at 1 January 1937		130 14 6
To Cambridge University Press		1330 10 1
To Copies bought in		13 10 0
		£1474 14 7

Cr. — INCOME

	£ s. d.	£ s. d.
By Sales—Current Volume		600 16 6
By Sales—Back Volumes, Parts and Sets		66 16 3
By Sales of Reprints		208 8 6
By Estimated Value of Stock at 31 December 1937		189 19 0
By Balance, carried down		408 14 4
		£1474 14 7

GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1937

Dr. — EXPENDITURE

	£ s. d.	£ s. d.
To *Annals of Applied Biology*, balance brought down		408 14 4
To Printing and Stationery		13 12 4
To Postages and Cheque Stamps		8 16 4
To Honorariums		6 3 0
To Subscription—Parliamentary Science Committee		10 10 0
To Sundry Out-of-Pocket Expenses of Secretaries and Treasurer		12 3 7
To Audit Fee Reserve		4 4 0
		£464 3 7

Cr. — INCOME

	£ s. d.	£ s. d.
By Members' Subscriptions:		
Arrears	23 15 0	
Entrance Fees	7 7 0	
Current	306 5 0	
		337 7 0
By Interest on National Savings Certificates and Bank Deposit		26 10 5
By Balance, being Excess of Expenditure over Income for the year		100 6 2
		£464 3 7

BALANCE SHEET 31 DECEMBER 1937

LIABILITIES AND SURPLUS

	£ s. d.	£ s. d.
Sundry Creditors:		
Cambridge University Press	467 18 10	
Audit Fee Reserve	4 4 0	
Sundry Expenses	17 16 8	
		489 19 6
Subscriptions and Entrance Fees paid in advance		12 6 0
Excess of Assets over Liabilities:		
As Balance Sheet of 31 December 1936	953 4 9	
Less: Balance of Income and Expenditure Account for 1937	100 6 2	
		852 18 7
		£1355 4 1

ASSETS

	£ s. d.	£ s. d.
Cash:		
At Bank on Current Account	74 0 1	
At Bank on Deposit Account	250 0 0	
		324 0 1
Debtors for Subscriptions, 2 years or less in arrear and considered good		60 0 0
500 National Savings Certificates		781 5 0
Stock of *Annals of Applied Biology* at estimated value		189 19 0
		£1355 4 1

J. HENDERSON SMITH, *Hon. Treasurer.*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association.

(Signed)
H. J. COX & CO.
Incorporated Accountants } *Auditors.*

HARPENDEN, 8 *February* 1938.

VOLUME XXV AUGUST, 1938 No. 3

THE VIABILITY OF WEED SEEDS AT VARIOUS STAGES OF MATURITY

By N. T. GILL, B.Sc., Ph.D.

Midland Agricultural College, Sutton Bonington, Loughborough

(With 4 Text-figures)

Introduction

The practice of cutting down plants before seed is produced is recommended for the eradication of certain weeds, with the precaution that cutting should be done early in order to prevent the maturation of seed on the cut shoots lying on the ground. Certain plants appear to mature their seeds after being cut, but whether these seeds are capable of germination is uncertain in many cases.

More detailed information on the stage of growth at which weeds are capable of producing viable seeds on the cut shoots is desirable, not only in connexion with the cutting of large weeds during weeding operations but, also, in connexion with the haying of grass containing weed species, and the cultivation of arable land. Fleischmann (1928) has shown that viable seed can be obtained from wheat if this is harvested after about one-third of the period between fertilization and the time at which maturity would normally occur. The object of the present investigation was to determine the time after which weeds, when cut, would similarly produce viable seeds which might find their way to the soil and germinate.

Methods

Common weeds were cut down near the base of the plant and allowed to dry in the sun. Samples were obtained at stages of development of each species varying from that at which the flower was still in the bud, through the open-flower stage, to various stages of maturity of the seed after fertilization. Samples of "dead-ripe" seed matured on the growing plant were obtained from each species for comparison. All samples were taken from at least ten distinct plants of each species, except in the case of thorn apple (*Datura Stramonium* L.), of which only seven plants were obtained. The seeds of each stage of maturity so obtained were mixed before testing. Germination tests were made using 100 seeds on

damp filter papers in Petri dishes, each test being performed in duplicate. The tests were usually made in an incubator at a temperature of 20–22° C. The fact that some seeds germinate better in the light and with fluctuating temperature (Warington, 1936) was taken into account, and, in some cases where germination was slow in the incubator, more rapid germination was obtained by standing the dishes on a laboratory bench near a window under conditions of fluctuating temperature and illumination.

With some species germination did not reach a high percentage until after a period of storage; therefore the seeds of these plants were stored in glass bottles in an open garden shelter and tested periodically until germination occurred freely or, in the case of the immature seeds, until it became apparent that germination would not occur.

As the object of the investigation was to demonstrate the viability of seeds harvested at various stages of development, and not to determine the maximum germination possible for the samples, tests were in some cases discontinued after a germination capacity of 50% or more had been demonstrated, although it is possible that, if such samples had been tested at later dates, higher figures would have been obtained.

Observations

(i) *Plants belonging to the family Compositae*

Many of the more common weeds belonging to the family Compositae were obtained at well-defined stages of growth:

(*a*) When all the flowers in the capitulum were still in bud.

(*b*) When the bifid stigmas were open.

(*c*) When the corolla was dying and the pappus beginning to extend.

(*d*) When dead-ripe fruits had formed.

It has been shown by La Rue (1935) that dandelions (*Taraxacum vulgare* Schrank.) may be cut at all stages of growth until the pappus begins to extend and, if the cut plants are left drying on a lawn, they will not produce seeds. Our experiments with this species gave similar results, but, within the family as a whole, results varied considerably.

The results of the germination tests of the species examined are summarized in Table I. For each species the figures were obtained by a test made within 2 weeks after collection of the seeds.

Ragwort. Providing the flowers were open and the stigmas visible viable seed was produced. When cut in the bud condition many of the buds on drying produced a white fluffy mass of pappus, but the "seed" attached was not capable of germination.

Table I

Germinability of seeds of Compositae

	Percentage germination		
	Dead ripe	Cut in flower	Cut in bud
Ragwort (*Senecio Jacobaea* L.)	72	80	0
Sow thistle (*Sonchus oleraceus* L.)	100	100	0
Groundsel (*Senecio vulgaris* L.)	90	35	0
Sea aster (*Aster Tripolium* L.)	90	86	0
Dandelion (*Taraxacum vulgare* Schrank.)	91	0	0
Cat's ear (*Hypochaeris radicata* L.)	90	0	0
Creeping thistle (*Cirsium arvense* Scop.)	38	0	0

Common sow thistle. All open flowers appear to be capable of producing viable seeds on drying.

Groundsel. A small but, from a weed infestation point of view, a very appreciable proportion of the seeds produced by the open cut flowers are capable of germination. The percentage of the seeds germinating in this sample did not increase during storage.

Sea aster. Although not of importance as a weed this species was included for comparison with other pappus-bearing species. The flowers ripened their seeds after the plant had been cut down.

Dandelion. Whilst ripe seeds gave a high percentage germination, flowers, when cut down, unlike *Senecio* did not produce any viable seeds, and the pappus did not elongate.

Cat's ear. A common weed in hay in some districts, gave results similar to dandelion.

Creeping thistle. The germination capacity, even of ripe seeds, was low and remained so even after storage. Very few fruits were produced by plants cut down when the flowers were in bloom, and none of these was capable of germination. Although, in external appearance, they were like the normally ripened fruits, they consisted almost entirely of an empty pericarp without any fully developed embryo.

A preliminary examination of spear thistle (*Cirsium lanceolatum* Scop.) showed that the plants cut in the flowering condition produced few fruits and that these, although looking like normal fruits, did not contain a mature embryo and would not germinate.

(ii) *Plants belonging to the family Gramineae*

Two grass species common in meadows and, therefore, frequently present in hay were examined, namely meadow barley grass (*Hordeum nodosum* L.) and soft brome grass (*Bromus mollis* L.). In each case plants cut in the flowering condition (i.e. when stigmas were visible)

did not ripen their grains, but plants cut after the stigmas had withered when the grains were in the milk-ripe condition (in each case the plants were still green at this stage) produced seeds with a high percentage germination. The percentage figures obtained within 2 weeks of harvesting the plants were:

	Dead ripe	Milk ripe
Meadow barley grass (*Hordeum nodosum* L.)	94	90
Soft brome grass (*Bromus mollis* L.)	96	81

(iii) *Other species*

Curled dock (Rumex crispus *L.*).

The plants of this species were cut down in September, but it was not until 3 months later that any of the seeds obtained would germinate in the incubator, although it has since been found that the seeds will germinate within a month under conditions of fluctuating temperature and illumination. Plants cut in the flowering condition did not ripen seed, but plants in which the seed had begun to form and was in the milk-ripe condition ripened viable seeds. On such plants the perianth around the fruits was still green and the leaves had not begun to die.

The percentage figures obtained for germination in the incubator after a period of dormancy lasting for 3 months were:

Dead-ripe seeds	...	84
Milk-ripe seeds	...	88

Thus, it is advisable to cut this plant very early before the seeds have begun to form.

Preliminary tests on broad dock (*Rumex obtusifolius* L.) indicate that this species behaves in a similar manner.

Thorn apple (Datura stramonium *L.*).

Although only a casual weed this plant has yielded interesting results and was, therefore, thought worthy of inclusion. The results of the tests are shown in Fig. 1. Dead-ripe seeds, immediately after collection from the dried plant, gave 100% germination. From the partly ripe green capsules present on the plant, 35% of the seeds collected were green and wrinkled and incapable of germination; the remainder were black like the normally ripened seeds, and their germination capacity immediately after drying was 67%. A month later the germination capacity of the dead-ripe seeds was nil, whilst the germination capacity of the black seeds from the immature capsules had reached 100%. The seeds were tested at intervals throughout the winter and the following summer, and the germination capacity of the "immature seeds" remained at 100%,

whilst that of the mature seeds remained nil. It was found, however, that when the seed coat of the mature seeds was first cracked 100% germination could be obtained. Thus, it seems that if dead-ripe seeds are placed under conditions suitable for germination immediately after being shed they germinate freely but, after a few weeks, the seed coat of ungerminated seeds becomes impermeable and the seeds assume a

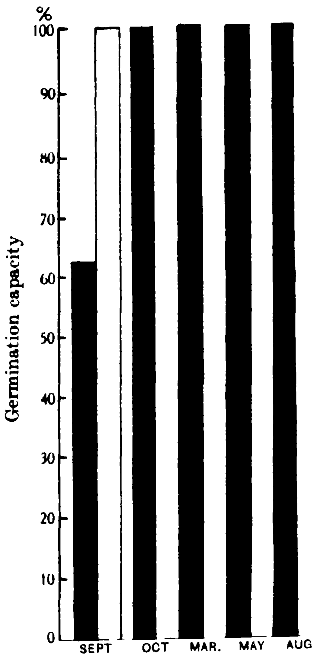

Fig. 1.

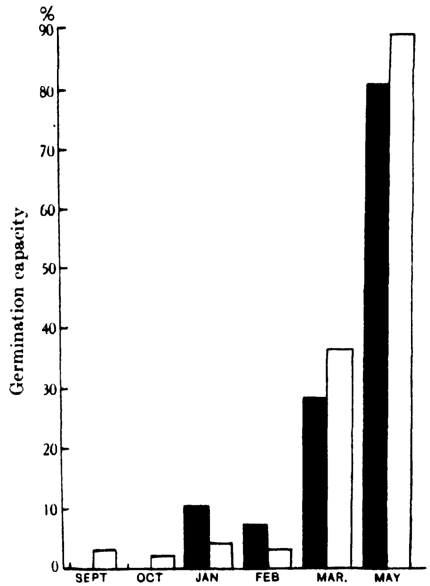

Fig. 2.

Fig. 1. Germination of stored seeds of thorn apple (*Datura stramonium* L.) collected in September. □ dead-ripe seeds; ■ seeds from immature green fruits.

Fig. 2. Germination of stored seeds of shepherd's purse (*Capsella Bursa pastoris* Med.) collected in September. □ dead-ripe seeds; ■ seeds from sun-dried large green fruits.

dormant condition. The seed coat of the "immature seeds", on the other hand, does not become impermeable and germination continues freely.

Shepherd's purse (Capsella Bursa pastoris *Med.*).

The seed capsules were divided into four groups: dead ripe, large green, medium green, and small green. The last two groups did not produce any viable seeds, but the first two both produced seeds capable

of germination after remaining dormant through the winter. The results are shown in Fig. 2. There was little germination of either the ripe seed or the immature seed until March and in May the germination of each was high and vigorous.

Corn speedwell (Veronica agrestis *L.*).

As in the case of the last species only the largest of the immature fruits produced viable seeds, and the germination of these was closely

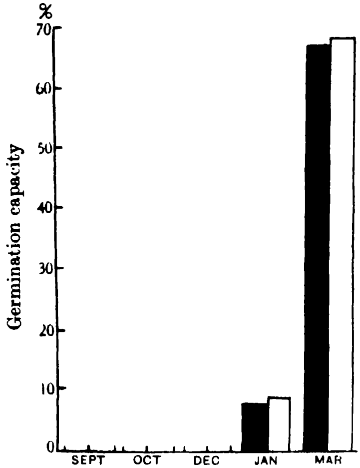

Fig. 3.

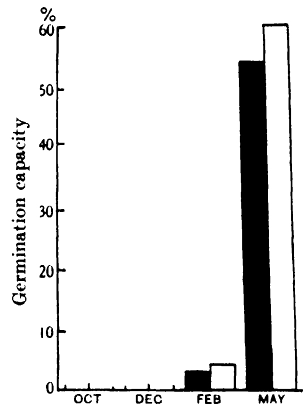

Fig. 4.

Fig. 3. Germination of stored seeds of corn speedwell (*Veronica agrestis* L.) collected in September. □ dead-ripe seeds; ■ seeds from sun-dried large green fruits.

Fig. 4. Germination of stored seeds of common chickweed (*Stellaria media* Vill.) collected in September. □ dead-ripe seeds; ■ seeds from sun-dried green fruits.

comparable with that of the dead-ripe seeds. In each case seeds collected in September did not give vigorous germination until March (Fig. 3).

Common chickweed (Stellaria media *Vill.*).

The flowers did not produce seeds after being cut down, but any green immature fruits present at the time of cutting ripened seed capable of germination, like the dead-ripe seeds, after a period of dormancy through the winter (Fig. 4).

Poppy (Papaver dubium *L.*).

Dead-ripe seeds were tested regularly for a year after collection but did not germinate, whilst seeds from green sun-dried capsules collected

in September, after remaining dormant in the winter, gave an appreciably large germination in February and throughout the summer.

The percentage figures obtained were:

	Sept.	Oct.	Dec.	Feb.	May	Aug.
Dead-ripe seeds	0	0	0	0	0	0
Seeds from immature green capsules	0	0	0	46	36	44

Thus, it would appear that seed from immature green capsules is capable of producing new plants in the season after its production, whilst dead-ripe seed passes into a dormant condition. It should be added that some of the dead-ripe seed was sown in soil in pots placed outside, and this failed to germinate in the first year. Exposure of samples, during germination tests, to fluctuating temperature and illumination failed to encourage germination in the dead-ripe samples, or to increase the germination capacity of the "immature green" samples. As in the case of *Datura*, when the seed coat of the dead-ripe seeds was first cracked, germination occurred freely.

Common nettle (Urtica dioica *L.*).

Samples cut in the flowering condition when the stigmas were showing did not ripen seed, but plants cut when the seeds were in the milk-ripe condition, and the perianth still fresh and green, ripened seed of high germination capacity. These seeds, together with the dead-ripe seeds, showed low germination capacity during the first month after collection, but, a month later, germination in both cases was vigorous. The percentage figures obtained were:

	Sept.	Nov.
Dead-ripe seeds	27	77
Milk-ripe seeds	22	70

Discussion

It is impossible to make any natural grouping of plant species with regard to the ripening or non-ripening of their seeds when the plants are cut in an immature condition. Within the family Compositae alone some species ripen seeds when cut down in the flowering condition; others produce apparently normal fruits which, however, contain no germinable seed; whilst others merely shrivel, making no progress toward the production of seeds. In the species examined in other families it seems to be necessary for the seeds to be present in the milk-ripe state before the plant is cut down if viable seeds are to be produced.

In the majority of species examined the germination of seeds harvested in the early stages of development occurs at about the same time as that of dead-ripe seeds and, in species in which the dead-ripe seeds pass through a period of after-ripening before germination is possible, the "immature" seeds undergo a similar period of after-ripening. *Papaver dubium* behaved in abnormal manner in this respect, the "immature" seeds beginning to germinate after 4 months' storage, whilst untreated dead-ripe seeds failed to germinate during the 12 months of the experiment. The fact that the dead-ripe seeds germinate if the seed coat is split indicates that dormancy is due only to the condition of the seed coat, and it is probable that further tests would show that the physiological after-ripening of the embryo of dead-ripe seeds takes about the same length of time as that of the "immature" seeds, and that continued dormancy is enforced by the condition of the seed coat. Takiguti (1930) has demonstrated a similar effect in rape seed (*Brassica napella*), in which seed harvested in the milk-ripe state germinated freely, whilst seed obtained after the brown pigment had developed in the seed coat germinated in the incubator only if the seed coat were first removed. The ripe seed in this case could, however, be induced to germinate by submitting it to fluctuating temperature conditions during germination. Fluctuating temperature and illumination appeared to have no effect on dead-ripe *Papaver* seeds, but it is possible that variations of temperature other than those occurring outside, in the case of the seeds sown in plant pots, or in the laboratory in the case of the Petri dish tests, may bring about germination of the dead-ripe seeds within 12 months. *Datura* is similar to *Papaver* in behaviour, save that the embryo is capable of growth soon after harvesting and, in the case of the dead-ripe seeds, the changes in the seed coat which induce dormancy occur a short time after the seeds are shed.

The effect of fluctuating temperature and illumination on the germination of the various species has not been examined in detail, since the object of the investigation was to ascertain the viability of "immature" seeds. Only in cases where germination was very low in the incubator have variable conditions of temperature and illumination been tested. Gardner (1921) has already shown that light encourages the germination of *Rumex crispus*. Warington (1936) has shown a beneficial effect of fluctuating temperature combined with illumination on several weed species, and suggests that fluctuating temperature is probably the more important; this would appear to be supported by Gassner's experiments (1930) which show that many seeds which are aided to germinate

by light also germinate readily if submitted to intermittent temperature in darkness. Fluctuating temperature and light together were found to have a beneficial effect on both *Rumex* species; these, however, also germinated freely in the incubator after a short period of storage. Other species tested and found difficult to germinate in the incubator have not responded to fluctuating temperature and illumination. Some species, such as hemp nettle (*Galeopsis speciosa* Mill.), knotgrass (*Polygonum aviculare* L.), and corn marigold (*Chrysanthemum segetum* L.), have been tested periodically for a period of 12 months, and neither "immature" nor dead-ripe seeds have germinated. These species may undergo a prolonged after-ripening, or the conditions under which the tests were made may have been unsuitable for the germination of their seeds.

Brenchley & Warington (1930) found an apparent periodicity in the germination of seeds of various weed species present in field soil and consisting of seeds of unknown age. The tests performed on stored seeds have not been continued for a sufficiently long period to show whether such periodicity exists in seeds of any one harvest year under these storage conditions.

SUMMARY

1. Certain plants within the family Compositae when cut down in the flowering condition produce viable seeds; others do not. The first group includes: *Sonchus oleraceus*, *Senecio Jacobaea*, *S. vulgaris* and *Aster Tripolium*, whilst the second group includes: *Taraxacum vulgare*, *Hypochaeris radicata*, *Cirsium arvense* and *C. lanceolatum*.

2. Certain species in other families produce viable seeds when cut down at various stages of maturation of the fruits following fertilization.

3. When the mature seeds exhibit a prolonged dormant period, the seeds harvested in an immature state exhibit a similar period of dormancy.

4. In *Papaver dubium* and *Datura stramonium*, the immature seed germinated more readily than the fully ripened seed, owing to the impermeability of the seed coat of the latter.

5. A combination of fluctuating temperature and illumination improved the germination of the two *Rumex* species when tested soon after harvesting, but had no effect on other species found difficult to germinate in an incubator at constant temperature. Three months after harvesting of the *Rumex* species the seeds germinated equally well in the incubator at constant temperature.

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THE DEVELOPMENT OF SAINFOIN IN ITS SEEDING YEAR

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(With Plates XVIII and XIX and 7 Graphs)

CONTENTS

Introduction

Cultivated sainfoin is an agricultural crop of some importance in England, particularly on the chalky soils of the south and east where it is indigenous. Unfortunately, the acreage grown is unknown, as in the Ministry of Agriculture's statistics it is included in "rotation grasses and clovers". It is probably true that it is grown less at the present time than formerly, but it is still highly valued for pasture, hay and forage. There is some confusion as to its nomenclature, but it can best be defined as *Onobrychis sativa* Lam. (*sensu stricto*). Two varieties are in common use, common or single-cut sainfoin, *O. sativa* var. *communis* Ahlefeld, and giant or double-cut sainfoin, *O. sativa* var. *bifera* Hort. Common sainfoin is a long-lived plant which reaches its maximum yield in its third year. Leys of 20 years have been recorded, but usually 7 or 8 years is considered satisfactory, the end of a ley being determined by the invasion of weeds. Common sainfoin produces flowering stems in May or June and the ley can then be cut for hay. In its subsequent growth rosettes of leaves are formed but no stems, hence the aftermath can be used for grazing only. Giant sainfoin is a shorter lived plant and is used for short leys of 1 or 2

years. After cutting, it again sends up flowering shoots, giving a second cut of hay and, sometimes, it may flower a third time and give a third hay crop. A third variety has been described under the name of three-cut sainfoin characterized by thrice flowering, but the distinction is not constant and this form should be included in the giant variety (Zade, 1933).

In order to avoid excessive repetition, common sainfoin (*O. sativa* var. *communis*) will be referred to throughout this paper as "common", and giant sainfoin (*O. sativa* var. *bifera*) as "giant".

In spite of its agricultural importance, sainfoin has received little scientific attention as compared with other forage crops such as lucerne and clover. Little is known, for example, of the differences between the two varieties, giant and common, or of the development of sainfoin from seedling to mature plant. Our present knowledge of its seeding year behaviour may fairly be summarized as follows:

After the kidney-shaped cotyledons reach the soil surface, foliage leaves with various numbers of leaflets are produced. The first foliage leaf is simple, the second and third are trifoliate, and the later leaves are pinnate with from six to twelve pairs of leaflets and a terminal one. Short lateral branches are formed and the plant forms a rosette which tends to be more prostrate in common than in giant. Later, branches may elongate and bear inflorescences (Percival, 1936; Robinson, 1937; Fleischmann, 1932; Rees, 1928, 1931, 1932). According to Rees, the amount of flowering in the seeding year is variable and depends partly on the variety and partly on the time of sowing. The present work was undertaken with a view to filling in some of the details of this picture by a close study of the growth of sainfoin plants in their seeding year, with particular reference to differences between the giant and common varieties.

Experimental methods

The work was carried out at the University of Reading's Agricultural Botanic Garden at Shinfield, Berks. Samples of giant and common sainfoin were sown in rows in the open in 1935 and 1937 and kept under observation throughout each season. In 1935 the sowing date was 29 March, but in 1937, owing to the failure of an earlier sowing, the date was 18 May. A small quantity of seed was also sown in an unheated glasshouse in February 1937. Altogether, thirteen lots of giant and thirteen lots of common were sown. Counts were made of the number of leaflets in successive foliage leaves of the seedlings up to the appear-

ance of the sixth leaf. Beyond this it was impossible to determine the order of the leaves owing to the development of lateral branches. The figures for leaflet numbers were obtained by combining the results from all the lots of seed sown in 1935 and 1937. For convenience, the number of leaflets in successive leaves is indicated by a group of numbers, e.g. if a seedling at the four-leaved stage has the combination 1.3.3.5, this means that the first leaf is simple, the second and third leaves have three leaflets each, and the fourth has five leaflets. In 1937, the number of tillers in 400 spaced seedlings was counted.

GERMINATION

In the case of milled seeds, i.e. true seeds, germination is of the epigeal type normally found in the Leguminosae. The radicle breaks through the testa and elongates; the hypocotyl then elongates, withdrawing the cotyledons from the testa and carrying them to the soil surface where they open out and lie flat on the surface. The cotyledons are thick and fleshy, kidney-shaped, with a flat upper surface and a convex lower surface, and turn dark green on exposure to light.

In the case of unmilled seed, i.e. seeds inside the indehiscent pericarp, the first sign of germination is the protrusion of the radicle through the pericarp as figured by Nobbe (1876). The radicle always emerges at the same point. On the surface of the fruit is a network of raised vascular ridges, and the radicle forces its way through the large mesh near the upper end of the ventral suture (Pl. XVIII, fig. 1). The swelling of the seed then causes the pericarp to split along the dorsal suture, and the hypocotyl elongates and carries the cotyledons out of the pericarp through this split (Pl. XVIII, fig. 1), leaving the testa within the pericarp. The ventral suture was never observed to split. In consequence of this method of germination, the radicle is encircled by the mesh of the tough vascular ridges and it may be some weeks before this is broken, with the result that a constriction is produced in the young root. When a seedling is pulled up at a later stage the pericarp can still be found firmly attached (Pl. XVIII, figs. 2, 3).

While the majority of unmilled seeds germinate in this way, a few are unable to break through the pericarp, but this does not necessarily prevent germination. In such cases, the swelling of the seed splits the dorsal suture and the radicle can grow out through the split. The seedling is thus able to free itself completely from the pericarp.

In 1935 the cotyledons had appeared above ground by 23 April, 26 days after sowing. This is rather a long time, but conditions of tilth

and weather were unfavourable. Rees (1931) found that when seed was sown at the end of March, the seedlings appeared within a week.

The phenomenon of delayed germination is well known in grasses and clovers and, in view of its importance in the case of seeds sown for long leys, it is interesting to know whether or not common sainfoin exhibits this trait. Delayed germination in sainfoin was observed 200 years ago by Jethro Tull (1733), who found that if sainfoin was sown with a nurse crop many seeds did not germinate till the following spring. Rees (1931) found indications of it in his experiments and, in the present work, definite evidence was found. Seed was sown in May 1937 and fresh seedlings continued to appear until October, and again from February onwards the following year, more abundantly in common than in giant. Unfortunately, beyond this statement of fact no data were obtained.

The first six foliage leaves

The first six foliage leaves all arose at the same level, between the cotyledons, the epicotyl remaining short. The dates of their appearance in 1935 were as follows:

	Date	No. of days after appearance of cotyledons
Cotyledons	23 April	0
1st leaf	4 May	12
2nd leaf	12 May	20
3rd leaf	25 May	33
4th leaf	1 June	40
5th leaf	11 June	50
6th leaf	25 June	64

In 1937 the date of sowing was later and growth was more rapid.

The first leaf is usually simple, but a number of seedlings were found with two, three or four leaflets. In common there was a slightly higher proportion of simple leaves than in giant (Table I, Graph 1). Giant had an average of 1·21 and common of 1·16 leaflets per leaf, but the difference is not significant. The first foliage leaf has a long erect petiole arising from between the cotyledons. In a simple leaf the blade is broadly ovate and in two- and three-foliate leaves the lateral leaflets are narrower. Seedlings with simple and two-foliate leaves are shown in Pl. XVIII, fig. 2.

In the majority of seedlings the second leaf was trifoliate, but leaves with one, two, four or five leaflets were also found (Pl. XVIII, fig. 3). There was little difference between the two varieties (Table I, Graph 2). Both had a preponderance of trifoliate leaves, but the proportion of five-foliate leaves was slightly higher and of simple leaves slightly lower

in giant. The mean number of leaflets per leaf in giant, 3·04, was slightly but significantly greater than in common, 2·95.

Table I

Percentage frequency of leaflet numbers in the first six foliage leaves of sainfoin seedlings

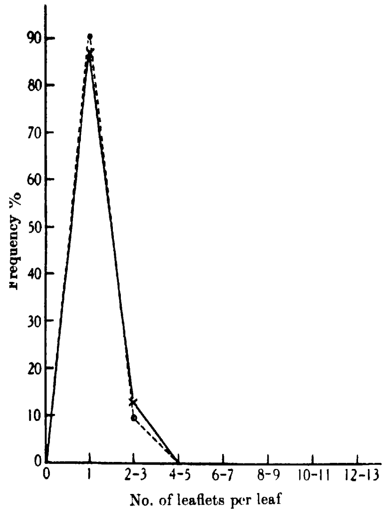
% frequency

No. of leaflets	1st leaf Giant	1st leaf Common	2nd leaf Giant	2nd leaf Common	3rd leaf Giant	3rd leaf Common	4th leaf Giant	4th leaf Common	5th leaf Giant	5th leaf Common	6th leaf Giant	6th leaf Common
1	87·1	90·3	0·7	1·8	0·1	0·4	—	—	—	—	—	—
2	4·4	3·2	3·1	4·6	0·8	1·1	0·2	0·1	—	—	—	—
3	8·4	6·5	91·0	91·6	57·2	72·7	19·2	41·5	3·2	13·6	0·2	1·5
4	—	Occ.	1·4	0·8	6·8	5·2	4·9	6·1	1·3	2·7	0·2	0·6
5	—	—	3·8	1·3	32·6	20·2	54·0	44·0	41·4	54·5	13·7	36·1
6	—	—	—	—	9·8	Occ.	3·1	1·2	4·4	2·7	1·2	3·4
7		—	—	—	1·6	9·4	14·9	6·1	29·2	19·7	35·8	35·5
8		—	—	—		—	0·5	0·1	2·4	0·8	3·7	1·9
9	—	—	—		—	—	3·0	0·9	12·6	4·8	28·4	14·5
10	—		—	—	—	—	0·1	—	0·6	0·4	1·9	0·7
11	—		—		—	—	0·1	—	4·3	0·8	14·4	4·0
12			—				—	—	—	—	Occ.	0·1
13	—	—	—	—	—	—	—	—	—	—	0·5	1·4
14	—	—	—	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—	—	Occ.	0·2
Mean	1·21	1·16	3·04	2·95	3·80	3·46	4·48	4·28	6·40	5·40	7·99	6·75
S.E.	0·016	0·014	0·011	0·010	0·023	0·020	0·036	0·030	0·044	0·038	0·052	0·051
No. of leaves counted	1326	1459	1756	1858	1910	1923	1840	1803	1696	1652	1327	1352

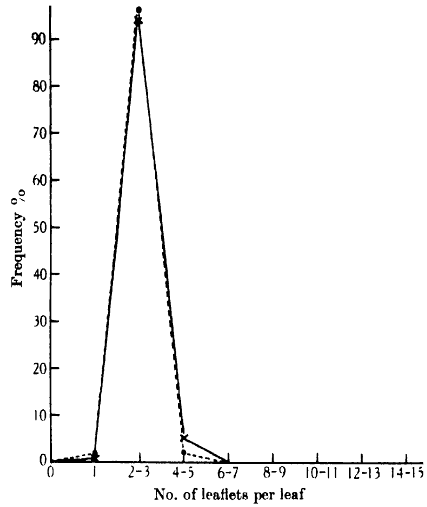
Third leaves with three and five leaflets were most frequent, but there were also leaves with one, two, four, six and seven leaflets (Pl. XVIII, fig. 4, Table I and Graph 3). A definite difference between giant and common seedlings had at this stage become apparent, the former tending to have a greater number of leaflets. Giant had 57·2% of its leaves trifoliate and 32·6% five-foliate, while the corresponding percentages for common were 72·7 and 20·2. The mean number of leaflets per leaf in giant was 3·8 and in common 3·46, the difference being significant.

In the fourth leaf, three and five leaflets were again most usual, but leaves with leaflets varying from two to eleven were found (Pl. XVIII, fig. 5, Table I and Graph 4). The difference between giant and common was maintained. While giant had a higher proportion of leaves with five or more leaflets, common had a higher proportion with less than five. The mean number of leaflets per leaf was again significantly different, giant having 4·48 and common 4·28.

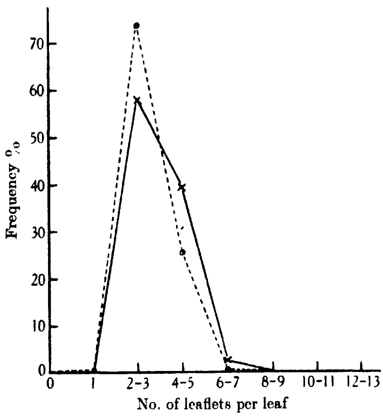
The number of leaflets in the fifth leaf varied from three to eleven, but the most frequent numbers were five and seven (Pl. XVIII, fig. 6,



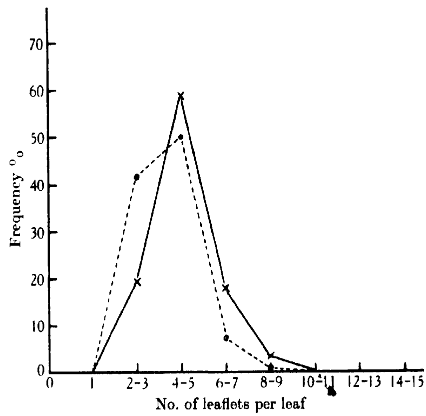
Graph 1. First leaf.



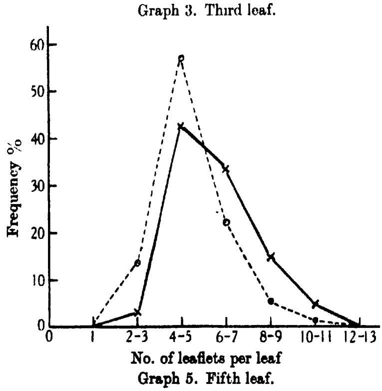
Graph 2. Second leaf.



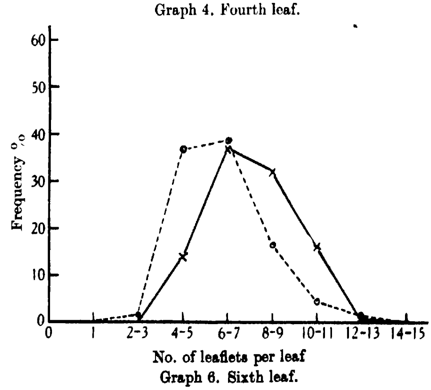
Graph 3. Third leaf.



Graph 4. Fourth leaf.



Graph 5. Fifth leaf.



Graph 6. Sixth leaf.

Table I and Graph 5). The higher numbers were more frequent in giant than in common. Giant had an average of 6·4 leaflets per leaf and common 5·4, the difference again being significant.

The number of leaflets in the sixth leaf was found to vary from three to fifteen, the most usual numbers being five, seven and nine (Pl. XIX, fig. 7, Table I and Graph 6). In both giant and common the most frequent number was seven, but common had a higher proportion of leaves with less than seven leaflets than giant. The mean numbers of leaflets per leaf were 7·99 for giant and 6·75 for common; the difference is significant.

From these figures it is evident that, as between individual plants, there is very considerable variation in the number of leaflets in each leaf. For example, the number of leaflets in the sixth leaf varied from three to fifteen. The most frequent combinations were 1.3.3.5.5.5 and 1.3.3.5.5.7. Other frequent combinations were 1.3.3.3.5.5, 1.3.3.3.5.7, 1.3.5.5.7.7 and 1.3.3.5.7.9. These, however, are only a few of the innumerable combinations actually present.

With the exception of the first leaf, giant had a significantly higher number of leaflets per leaf than common. It is doubtful, however, if much value can be placed on the actual mean figures obtained, even though the differences were significant statistically, as the variation between different samples examined was so great. Table II gives the average number of leaflets per leaf for the twenty-two samples in which counts were made. As an example, the average number of leaflets in the sixth leaf varied from 6·7 to 9·7 in giant and from 5·9 to 8·7 in common for different lots and, taking individual plants, the number varied from three to fifteen. The statement that the average numbers of leaflets in the sixth leaf of giant and common are 7·99 and 6·75 respectively is, in these circumstances, meaningless, and the only conclusion that may be drawn is that there is a tendency for giant seedlings to have a greater number of leaflets in each leaf than common seedlings.

It will be noticed that leaves with even numbers of leaflets appeared consistently. A normal leaf has an equal number of leaflets on either side, more or less paired, and a terminal leaflet. In leaves with an even number of leaflets, either one of the laterals or the terminal leaflet is missing.

Graphs 1–6. Percentage frequency distribution of leaflet numbers in successive foliage leaves. —— giant; - - - - - common.

Table II

Mean number of leaflets in the first six foliage leaves of different lots of sainfoin seedlings

Sample	Mean number of leaflets					
	1st leaf	2nd leaf	3rd leaf	4th leaf	5th leaf	6th leaf
Giant A	1·4	3·0	4·1	5·8	7·7	9·7
B	1·6	3·0	4·1	5·8	7·6	9·6
C	1·6	3·1	4·3	5·5	7·0	9·0
E	1·7	3·2	4·5	5·9	7·6	9·3
F	1·9	3·3	4·8	6·2	7·9	9·5
G	2·1	3·2	4·5	6·1	7·9	9·7
J	1·1	3·0	3·4	4·3	5·4	7·2
K	1·2	3·0	3·4	4·2	5·2	6·7
L	1·4	3·0	3·3	4·7	6·0	8·0
M	1·3	3·1	3·9	5·4	6·5	8·5
N	1·7	2·5	3·3	4·7	6·0	7·5
Common A	1·4	2·8	3·9	5·3	6·9	8·5
B	1·5	3·1	3·7	4·6	5·7	6·7
C	1·4	3·0	3·6	4·6	5·7	6·7
F	1·5	3·0	3·9	4·9	6·0	7·3
G	1·4	3·0	4·0	5·2	6·5	8·1
H	1·5	3·2	4·5	5·7	7·2	8·7
J	1·1	2·9	3·1	3·7	4·7	6·0
K	1·1	2·9	3·7	4·4	5·3	6·5
L	1·4	2·9	3·2	4·7	6·1	7·8
M	1·0	2·9	3·2	3·8	4·7	5·9
N	1·3	3·1	2·8	4·4	4·9	7·0

Subsequent growth

No further counts of leaflets were made after the sixth leaf. So far, there was little obvious difference between the two varieties, giant and common. In both varieties the internodes remained short and the leaves arose at the same level forming a rosette. Superficially, the plots of giant appeared more luxuriant, and the counts have shown that this was due to the greater number of leaflets per leaf in that variety. From this stage onwards, however, there was considerable divergence in the development of the two varieties.

(*a*) *Common sainfoin.* In common sainfoin the internodes remained short throughout the season and new leaves continued to be formed on the short main stem. Soon after the formation of the sixth leaf, lateral buds began to develop. These laterals produced numerous leaves but their internodes normally remained short, thus giving a more dense appearance and accentuating the rosette habit. An occasional tiller was found in which the internodes had elongated, producing a prostrate lateral branch up to 6 in. in length, but this was exceptional. The plants remained in this condition until the end of the season and never flowered.

Rees (1932) found that some lots of sainfoin, which purported to be of the common variety, flowered in the seeding year, particularly continental strains. His work suggests, however, that these were mixtures of giant and common types and that the true common does not flower in the seeding year. Of the thirteen lots of common, including one of French origin, sown at Shinfield none showed any flowering.

In the autumn two types of plant could be distinguished, prostrate and semi-erect. In the prostrate type the leaves of the main stem and of the tillers all lay close to the ground as shown in Pl. XIX, fig. 8. In the semi-erect type the leaves of the main stem and of the tillers were produced at all angles from horizontal to erect as shown in Pl. XIX, fig. 9. The two types are not absolutely distinct, but merge into each other. Nevertheless, a population of plants can be separated into the two types quite readily. The angle of the leaves of the tillers is determined by the angle at which the tillers leave the main stem. Counts made in September 1937 showed that 46·5% of the common plants were of the prostrate type, and 53·5% of the semi-erect type.

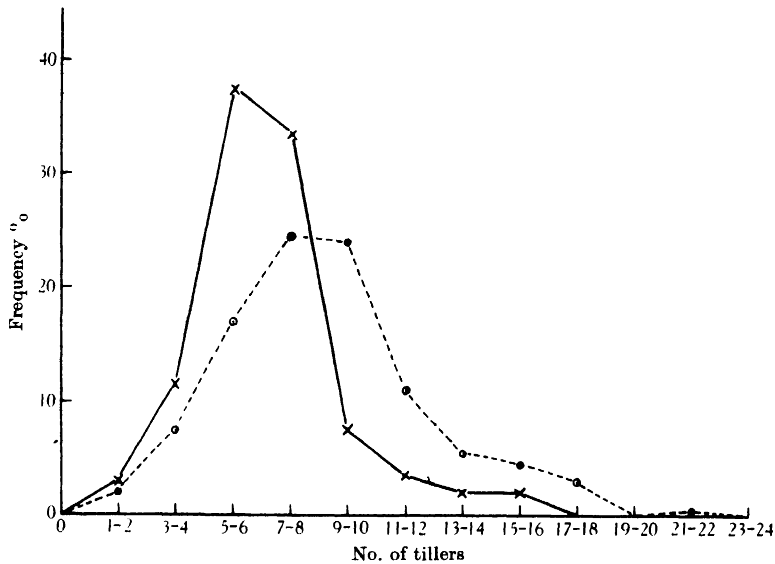
(*b*) *Giant sainfoin.* In giant sainfoin the subsequent behaviour was variable. In some plants the internodes remained short as in common, in others the internodes elongated and the plants assumed an erect habit. Most of these erect plants flowered. The rosette plants were similar to the semi-erect type of common (Pl. XIX, fig. 10). They behaved in the same way and did not flower.

The internodes of the erect giant plants began to elongate after the production of the sixth leaf. Elongation took place in the upper internodes, the first four to six internodes remaining short. Pl. XIX, fig. 7*a* shows an erect giant seedling with eight leaves, the first four arising at the same level. Lateral buds developed in the axils of the lower leaves and these tillers either elongated, or remained short and produced a tuft of leaves at the base of the plant. Pl. XIX, fig. 11 shows an erect plant with two lateral branches, one of which has elongated and the other has remained short. The elongated laterals frequently grow out horizontally for about an inch and then turn abruptly upwards. In the axils of the upper leaves either inflorescences or branches may be produced. The height of an erect plant is about 15 in.

The plots were cut on 21 August in 1935 and on 31 July in 1937. After cutting, lateral buds developed rapidly. In the case of the rosette plants, they retained this habit after cutting. In the case of the erect plants, the tillers either remained short producing a rosette habit, or else elongated giving an erect habit again.

Flowering in the seeding year is very variable. In 1935 flowering began on 1 July and only 3·3 % of the plants flowered. After cutting, all the plants assumed the rosette habit and none flowered. In 1937 the flowering date was 20 July and 93·5 % of the plants flowered. After cutting, practically all the plants produced erect stems and 94·5 % flowered a second time on 30 August.

This variability is reflected in the little information available as to the proportion of giant plants flowering in their seeding year. Fleischmann (1932) records that, in plots, 6 % of the plants flowered but,



Graph 7. Percentage frequency distribution of tiller numbers. —— giant; ----- common.

according to Klapp (1932), most of the sainfoin grown in Germany shows flowering in up to 100 % of plants. According to Rees (1928), giant usually flowers abundantly once or twice in its seeding year in South Wales, but flowering can be influenced by the time of sowing. If sowing takes place before the end of May the seedlings will flower but, if sowing is delayed till after May, flowering will be reduced or prevented. It is probable that flowering in the seeding year is influenced to a large extent by weather conditions. Sainfoin cannot be grown successfully in this country north of the Humber; therefore, even in the south of England, the conditions must be very close to the limits which sainfoin

will tolerate and, in a bad season, these limits may easily be exceeded. Cold or wet weather in spring or early summer, as in 1935, may so retard early growth as to prevent the plant reaching the flowering stage that season. In such circumstances it is impossible to give a figure which will represent the degree of flowering with any certainty.

NUMBER OF TILLERS

The number of tillers was counted in 200 seedlings of common and 200 of giant on 29 July 1937. The seedlings were spaced at 9 in. The results are given in Table III and Graph 7. The number of tillers varied from one to sixteen in giant and from two to twenty-one in common, the most frequent number in the former being six and in the latter nine. The mean number of tillers per plant in giant was 6·67 and in common 8·73, the difference being significant.

Table III

Percentage frequency of tiller numbers in sainfoin seedlings

	% frequency	
No. of tillers	Giant	Common
1	1·5	—
2	1·5	2·0
3	3·0	4·5
4	8·5	3·0
5	15·5	4·5
6	22·0	12·5
7	19·5	12·5
8	14·0	12·0
9	5·0	15·0
10	2·0	9·0
11	1·0	5·5
12	2·5	5·5
13	2·0	2·5
14	—	3·0
15	1·5	2·5
16	0·5	2·0
17	—	1·0
18	—	2·0
19	—	—
20	—	—
21	—	0·5
No. of plants counted	200	200
Mean no. of tillers	6·67	8·73
S.E.	0·1764	0·2482

This greater tillering power in common emphasizes the difference in behaviour between the two varieties. While giant produced tall stems and flowered, common remained prostrate and produced a greater number of tillers.

Discussion

Sainfoin usually is sown with a nurse crop, so that the course of development described above does not necessarily agree exactly with what actually happens in farming practice.

Giant and common sainfoin are taxonomically indistinguishable, and the recognition of a plant as belonging to one or the other variety depends upon growth behaviour. Giant grows more rapidly, producing two or even three cuts per season and is short-lived, while common will only give one cut followed by grazing and is long-lived. The account given above of the behaviour of sainfoin plants in their seeding year is consistent with this distinction, and the difference is exhibited from the beginning. In the first foliage leaf there is no significant difference between the varieties but, in the second to the sixth leaf, giant consistently has a significantly higher average number of leaflets than common. At the six-leaved stage the total number of leaflets averaged 26·9 for giant and 24·0 for common. The surface area of the leaflets was not actually measured, but observation and comparison have revealed no obvious difference between the varieties in this respect. If this be so, then the number of leaflets can be taken as an approximate measure of leaf area, and it follows that in giant the area of each leaf is greater than that of the corresponding leaf of common, and that the rate of increase of leaf area is greater in the giant variety. The rate of increase of number of leaves, however, was the same in both varieties, the appearance of corresponding leaves being almost simultaneous.

At the rosette stage common is more prostrate than giant and, at a later stage, giant tends to run to stem and flower while common retains the rosette habit and produces leaves only. Correlated with this prostrate habit of common is a greater tillering power.

It has been suggested by Koreisa (1935) that the difference in behaviour between the two varieties is due to a difference in the length of the thermo-stage in development. He divides perennial plants into two groups, viz.:

(1) Winter perennials. These are plants which, when sown in spring, do not flower the first season. They have a long thermo-stage and must pass through winter before flowering.

(2) Spring perennials. These plants have a short thermo-stage and, when sown in spring, flower the same season.

Common sainfoin belongs to the first group and giant sainfoin to the second. Temperature may certainly be a factor determining the frequency

of flowering, but to attribute all the differences between the varieties to temperature requirement would be an over-simplification.

The high degree of variability as between individual plants shows that commercial stocks of sainfoin are mixed populations. The frequency curves for numbers of leaflets are very similar in giant and common and there is considerable overlap between the varieties. This suggests that they both have been derived from the same original mixed stock by a process of mass selection. Unfortunately, there is no historical evidence as to the separation of the two varieties.

The distinctive features of giant sainfoin are:

(*a*) In the seeding year it tends to run to stem and flower.

(*b*) Its growth is more rapid and luxuriant throughout.

(*c*) It flowers again after cutting.

(*d*) It is short-lived.

Seed is harvested in the second or third year and this involves a selection in favour of plants which come to maturity quickly and are, therefore, probably short-lived. The seed is always taken from the second cut and consequently only from plants which flower a second time. In comparison with common, therefore, mass selection is for second flowering combined with early maturity.

The distinctive characters of common sainfoin are:

(*a*) In the seeding year the plants are prostrate and no stems or flowers are formed.

(*b*) Its growth is less rapid and luxuriant.

(*c*) No flowers are formed after cutting.

(*d*) It is long-lived.

The position here is complicated by the fact that two regional strains of common have been evolved. In the eastern counties, seed is taken in the early years of a ley, and plants grown from such seed tend to be non-persistent and to show some second flowering. In Hampshire and on the Cotswolds, seed is harvested in the later years of a ley, and gives rise to plants which are long-lived and show no tendency to second flowering (Rees, 1932). Hampshire and Cotswold sainfoin is therefore of the true common type, while Eastern Counties sainfoin is intermediate between giant and true common. Rees shows that there is strong evidence of a correlation between longevity and once-flowering. This would suggest that the original selective factor separating the giant and common varieties from a common stock was the time at which the seed was harvested. By harvesting seed early or late in a ley the two varieties

would become differentiated by the association of flowering frequency and other non-selective characters. It would not be expected, however, that such varieties would be genetically pure and, consequently, there is found considerable overlap between them.

SUMMARY

1. Evidence of delayed germination was obtained.

2. The number of leaflets in each of the first six foliage leaves of sainfoin seedlings shows considerable variation. The mean number of leaflets in each of the second to the sixth foliage leaves is significantly greater in giant sainfoin than in common sainfoin.

3. Giant sainfoin tends to produce erect stems and flower in the seeding year, the amount of flowering being variable. Common sainfoin remains prostrate and never flowers.

4. Common sainfoin produces more tillers than giant sainfoin.

5. The origin of the giant and common varieties is discussed.

I wish to express my thanks to the following seed merchants for supplying samples of seed: Dunn's Farm Seeds, Ltd., Salisbury; Gartons, Ltd., Warrington; A. G. Leighton, Ltd., Whitchurch; Smith Bros., Ltd., Basingstoke; Suttons and Sons, Ltd., Reading; Edward Webb and Sons, Ltd., Stourbridge.

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orcadensis, No. 521; *A. brevis*, No. 2384. These selections have been multiplied every year by growing a few plants in pots at a distance from other oats. The propagation of the smuts on appropriate varieties is described later. Collection C_3, a duplicate of C_4, was discarded at an early date, and collection L_1 was lost after a few years, probably as a result of contamination by L_2. Since both were propagated on var. Potato the accident was not discovered until it was too late to trace its source and it is impossible to recover L_1 by screening. Fortunately L_{12}, a duplicate of L_1, had been kept in cultivation. Thus the six types recognized as distinct biological species in 1927 have been grown and studied for a period of 10 years, and additional information has been obtained concerning their behaviour and relative stability on the differential hosts.

In work with smut fungi the terms "biological species" or "physiologic race" have been applied, in most cases, to spore collections which have given distinctive and consistent results over a number of years. One of the authors used the term biological species in this sense in 1929 although recognizing (Sampson, 1929, p. 78) "that certain collections (e.g. L_2) which produce infection on varieties belonging to widely different species of the host may be a mixture of two or more biological species". In other words, a so-called biological species may be a population of distinct biotypes, which gives consistent results over a certain period of time. Ideally a biological species should consist of only one genotype. Following Resolution 14 of the Sixth International Congress, Amsterdam, 1935, it is proposed in future to substitute "physiologic race" for "biological species" used in earlier papers of this series.

There are three methods of treating spore collections of smut fungi to bring them nearer to the desired purity of type. One is the use of differential host varieties in screening experiments. Given a mixture of spores of two types such as L_{11} and L_{12} (Table I) it should be possible, *if hybridization does not take place*, to obtain a clear cut separation in *one* generation by dusting the grain of the two differential hosts *strigosa* and Potato. Each host is completely uncongenial to one of the smut types and screens it out of the mixture. If the two smuts hybridize freely and segregate on Mendelian lines, the effect of screening upon a mixed population will depend largely upon the behaviour of the heterozygous dikaryophyte. Even so, we might expect that screening would, in the long run, isolate types closely approaching the two parents. For further discussion on the possibility of natural hybridization in the oat smut fungi see p. 502 of this paper.

Table I

The behaviour of eight spore collections of oat smuts on selected differential hosts

Compiled from data published by Sampson (1929, Tables IV, V and VI) and from additional data. See also Sampson (1933, Table I)

	Ustilago Avenae (Pers.) Jens.			*Ustilago Kolleri* Wille*		
	L_1 and L_{12} Wales	L_2 U.S.A.	L_{11} Wales	C_1 Wales	C_2 U.S.A.	C_3 and C_4 England
A. sativa:						
Potato	+	+	0	0	+	+
Abundance	0	+	0	Not used for *U. Kolleri*		
Grey Winter	Not used for *U. Avenae*			0	+	+
A. nuda	0	+	0	0	+	S
A. strigosa orcadensis	0	S	+	+	+	0
A. brevis	0	0	+	+	0	0

0 no smutted panicles.
\+ completely susceptible, usually 90-100%.
S - slightly susceptible 15-30%.

* The specific name *U. Kolleri* is now adopted in place of *U. laevis* used in previous publications in conformity with the list published by the Plant Pathology Subcommittee of the British Mycological Society (*Trans. Brit. Mycol. Soc.* **14**, 140 (1929)).

The second method of purifying material is to isolate a single chlamydospore, grow it in culture and build up a collection of spores by inoculating seedlings of a suitable host. Theoretically this procedure does not of necessity lead to a genetically pure strain of the fungus since the parasitic phase of the pathogen is dikaryophytic, nuclear fusion occurring in the chlamydospore, segregation in the promycelium, while sporidial or hyphal fusion with the pairing of nuclei precedes invasion of the host

Table II

Hosts on which the spore collections were propagated, 1928–35

One year (1929) was missed, and in 1930 one-year-old spores were used. Since 1935 monospore lines have replaced the original spore collections

	Spore collections					
	Ustilago Avenae			*Ustilago Kolleri*		
Year	L_2	L_{11}	L_{12}	C_1	C_2	C_4
1928	Potato	*Strigosa*	Potato	*Strigosa*	*Nuda*	Grey Winter
1930	,,	,,	,,	,,	Potato	,,
1931	,,	,,	,,	,,	*Strigosa*	Potato
1932	*Nuda*	,,	,,	,,	,,	,,
1933	,,	,,	,,	,,	Potato	,,
1934	,,	,,	,,	,,	,,	,,
1935	,,	,,	,,	,,	,,	,,

(Dickinson, 1927, 1927–8; Popp & Hanna, 1935). The purity of the population ultimately arising from a single spore will depend, therefore, upon the genetical constitution of the chlamydospore selected. A third method which should give quicker results is to start the cultures from the paired sporidia of one chlamydospore.

The first two methods have been studied at Aberystwyth. The object of this report is to re-examine the pathogenicity of the 1927 spore collections in the light of these results and to see in what degree they approached pure-breeding populations.*

II. TECHNIQUE

(1) *Propagation of spore collections*

Each year since 1927 the spore collections have been propagated upon appropriate hosts, using the following technique. Shelled grains of each oat selection were dusted with spores, placed in rows between two sheets of damp blotting paper, which were made into rolls and placed in an incubator at 22° C. for 3 days. The seedlings were transferred to boxes of soil (usually heated to destroy stray grains of oats and weeds) and left in a cool glasshouse for some weeks. Later the boxes were placed in a trench in the open cage until smutted panicles were formed in the sheath. Before exsertion took place the boxes were taken back to the glasshouse and glazed bags were tied firmly over the stems so that the spores matured in the bag. When the plants were judged to be ripe the stems were cut, tied in bundles without removing the bags and stored in the laboratory. During the winter the spores were rubbed through fine sieves and transferred to clean jars. Two collections were never dealt with in the same room on the same day, and, at each step of the work, precautions were taken to prevent admixture of strains. It was not practicable to keep each collection in a separate glasshouse.

Apart from the loss of collection L_1, the technique proved satisfactory. The susceptible varieties chosen as hosts (Table II) invariably gave 80–100% infection, and two boxes of each lot, comprising 60–80 plants, yielded an adequate supply of spores for the experiments involved.

(2) *Screening experiments*

The necessary annual propagation may change a spore collection by sifting out certain types. In experiments particularly designed to test the effect of screening, a portion of the spore collection was used for the

* Only pathogenicity is dealt with. Some of the collections of *U. Kolleri* contain echinulate spores but this does not affect the argument of this paper.

inoculation of the selected variety, spores were harvested under a new reference number and grown on the same variety for several years in succession. Finally, a test was arranged by which the "new" collection could be compared with the "parent" collection on several differential hosts. The method is essentially like that followed by other workers (Dillon Weston, 1932; Nicolaisen, 1934).

(3) *Monospore lines*

Isolation of spores was undertaken in February 1934, using a modification of the method described by Dickinson (1926). The procedure involved the removal of dry spores from a glass slip and their transference to agar drops on sterile cover-slips. The whole of this process, and the subsequent germination and development of the young colonies, could be kept under direct observation, and all contaminations were immediately discarded. The young colonies were later removed to tubes or plates and kept until required.

In accordance with the experience of several other workers, germination of isolated chlamydospores was, at first, found to be poor, even though others from the same collections germinated and grew readily on ordinary dilution plates. Attempts were made to pick off spores in tap and distilled water, very dilute agar, and cane-sugar solutions of varying strengths, with very indifferent success, and it was not until a dilute solution of sodium carbonate was tried that satisfactory germination was secured.

Stock cultures were grown on 1% meat extract agar, but since a weak medium, namely, 0·3% Knop solution agar, was found particularly favourable for sporidial fusions, this was the medium used for inoculation experiments. Shelled grains of the host varieties were germinated on filter paper, and after 2 days, when the plumules were just commencing to grow, they were rubbed with sporidia from a culture which had been well mixed in order to increase the possibility of including sporidia of both sexes. A small piece of agar, bearing sporidia, was then cut out and left attached to the young seedlings, after which the grains were planted in moist soil in earthenware dishes, covered with small bell-jars to maintain a high humidity, and placed in an incubator at 22° C. The following day the process was repeated and the dishes left in the incubator for several days and then removed to window ledges at room temperature. When the seedlings were from 2 to 3 weeks old they were transplanted to pots and boxes in a cool glasshouse for further development. The method was tedious but gave fairly satisfactory results; 53%

of the total number of plants was smutted, and only four lines out of a total of thirty failed to reproduce spores. The electrical vacuum-pump method of inoculation described by Allison (1937) was also used successfully in some experiments. The chlamydospores obtained were tested in 1935 in the differential host varieties by the technique outlined above (p. 493), and additional confirmatory tests were made in 1936.

Since difficulty was experienced in obtaining germination of isolated single spores of C_4, a slight modification in technique was necessary for this collection. Since good germination was observed when the spores occurred on the agar in clusters, spores for isolation were smeared along one side of an agar drop and removed when the first indication of a promycelium could be seen. Subsequent growth was normal and by this method eight lines were obtained and studied.

(4) *Comparative growth of monospore lines in culture*

Having obtained several monospore lines of each spore collection in culture, experiments were designed to compare their cultural characteristics. The medium selected was similar to that used by Dickinson (1928). Its composition was as follows:

	gm.
Maltose	0·536
Urea	0·011
Dipotassium phosphate	0·500
Magnesium sulphate	0·250
Potassium chloride	0·250
Agar	1·500

Distilled water to make up 100 c.c.

Dipotassium phosphate was preferred to the acid phosphate used by Dickinson, and the pH was adjusted to 5·6 by means of dilute hydrochloric acid. All the cultures used for comparison were grown in dishes of the same size, and 7·5 c.c. of medium were introduced into each. This amount ensured an adequate supply of nutrient and, at the same time, left a relatively thin film of agar on the bottom of the container through which the details of growth and development could easily be studied. All cultures were grown at room temperature, and experiments designed to establish the presence or absence of individual characteristics of the spore collections, and the amount of variance between monospore lines within each one of them, were carried on for some months. As these were repeated several times and, where possible, set up in triplicate, it is considered that the results obtained present a true picture of the cultural behaviour of the different lines studied.

III. Presentation of results

(1) *Pathogenicity of the spore collections*

It is convenient to discuss each collection separately, recording only changes which may have taken place in the course of propagation or in connexion with the isolation and multiplication of monospore lines.

Collection L_2 was marked in 1927 by a wide range of infection attacking a number of *sativa* varieties and in addition selections of *nuda* and *strigosa*. L_2 is an American collection, and similar results were obtained with it at Missouri (Reed, 1929). Since 1927 it has been propagated either on Potato or *nuda* (Table II). Subsequent tests have shown that its virulence on these hosts is unchanged, that is, it is apparently not a mixture which can be separated into two types by propagation on these hosts. On the other hand, it has lost the power to infect *strigosa*. Five monospore lines were studied and they gave uniform results attacking Potato, Abundance and *nuda*, and failing on *strigosa* and *brevis* (Table III).

Table III

Inoculation of differential hosts with monospore lines,
1935–6. Ustilago Avenae

Ref. to monospore line	% infection: Potato (2855) 1935	Potato (2855) 1936	Abundance (2807) 1935	Abundance (2807) 1936	*A. nuda* (2495) 1935	*A. nuda* (2495) 1936	*A. strigosa* (521) 1935	*A. strigosa* (521) 1936	*A. brevis* (2384) 1935	*A. brevis* (2384) 1936
$L_{2/1}$*	17	—	4	—	15	—	0	—	—	—
$L_{2/2}$*	42	-	30	—	27	—	0	—	—	
$L_{2/3}$	78	—	64	—	69	—	0	-	-	
$L_{2/4}$	76	97	92	67	83	98	0	0	—	0
$L_{2/5}$	81	68	74	58	96	95		0	-	0
$L_{11/1}$	0	—	0	—	0	—	100	—	—	—
$L_{11/3}$	0	0	0	0	0	0	100	100	—	100
$L_{11/4}$	0	—	0	—	0	—	62	—	—	—
$L_{11/5}$	0	—	0	—	0	—	88	—	—	-
$L_{12/1}$	—	98	—	0	—	0	—	0	—	0
$L_{12/2}$	—	100	—	0	—	0	—	0	—	0
$L_{12/3}$	—	97	—	0	—	0	—	0	—	0
$L_{12/5}$	—	58	—	0	—	0	—	0	—	0

* Low infection with these lines was due to loss of viability in the chlamydospores.

Collection L_{11} has a limited host range attacking only selections of *strigosa* and *brevis* (Sampson, 1929, Tables IV and V). Repeated propagation on *strigosa* (at least 7 years) has not affected adversely its virulence on *brevis*, and four monospore isolations gave uniform and similar results (Table III). Further reference to the apparent purity of

this type will be found in connexion with the growth of monospore lines in culture (p. 501).

Collection L_{12} was propagated consistently on Potato. Four monospore isolations were made in 1935, multiplied on Potato and tested on five hosts in 1936 (Table III). Only Potato was infected, and the monospore lines differed consistently from L_{11} and L_2, and agreed with the original (1927) collection L_1 and the parent collection L_{12} in their inability to attack Abundance, *nuda*, *strigosa* and *brevis*.

Collection C_1. In regard to its infection capacity, this collection of *Ustilago Kolleri* is similar to *U. Avenae* L_{11}, attacking only *strigosa* and *brevis*. Five monospore lines gave uniform results (Table IV) infecting both *brevis* and *strigosa* heavily, although the parent collection had been propagated for six successive generations exclusively on the latter host.

Table IV

Inoculation of differential hosts with monospore lines,
1935–6. Ustilago Kolleri

Ref. to monospore line	% infection: Potato (2855) 1935	Potato (2855) 1936	Grey Winter (2860) 1935	Grey Winter (2860) 1936	*A nuda* (2495) 1935	*A nuda* (2495) 1936	*A. strigosa* (521) 1935	*A. strigosa* (521) 1936	*A. brevis* (2384) 1935	*A. brevis* (2384) 1936
$C_{1/1}$	0	0	-	0	—	0	100	100	100	100
$C_{1/2}$	0	—	—			—	100	—	100	—
$C_{1/3}$	0			—	–		100	—	100	—
$C_{1/4}$	0				–	—	100	—	100	–
$C_{1/5}$	0				—	—	89	—	68	—
$C_{2/2}$	89	96	–	34	--	89	95	99	0	0
$C_{2/3}$	100	79		41	—	77	100	98	0	1
$C_{2/5}$	93	95	–	71	—	90	0	0	0	0
$C_{4/1}$	75	100	—	84	0	0	0	0	0	0
$C_{4/2}$	80	100	—	82	70	100	0	0	0	0
$C_{4/3}$	83	96	–	50	96	100	0	0	0	0
$C_{4/5}$	75	98	—	78	91	93	0	0	0	0
$C_{4/8}$	—	93	–	—	—	0	—	—	—	—
$C_{4/12}$	–	93	–	-		0	—	—	—	—
$C_{4/13}$	–	93	–	—	—	0	—	—	—	—

Collection C_2, like the original L_2, attacked *strigosa*, *nuda*, and several varieties of *sativa* (Table I). It has been propagated on the three species since 1927 without any apparent screening effect. Of three monospore isolations made in 1934, two were uniform in behaviour and had the wide infection capacity of the parent collection, the third attacked Potato and *nuda*, but failed to attack *strigosa*. Since the infection experiment was repeated in 1936 with fully confirmatory results there seems to be little doubt that the monospore line $C_{2/5}$ is completely lacking in the power to infect *strigosa*. The significance of this result will be discussed

later in connexion with some isolations from C_4 which showed a somewhat similar change.

Collections C_3 *and* C_4[1] came originally from England on Grey Winter, and on this variety it has continued to give high infection. It also infects Potato with equal ease, and growth on either variety fails to change its virulence for the other host. In 1927 it seemed to have the property of infecting *nuda* to some extent. Including its duplicate C_3, five results were obtained in which an attack of 15–28% was recorded on *nuda*, and it became a matter of interest to know if it was a constant character of this collection to give a positive infection of this magnitude on *nuda*. If not, it might be expected that the degree of infection could be increased by growing for successive years on this host. The results shown in Table V were obtained.

Table V

Screening experiment with C_4 *on* Nuda

1930	1931	1932	1933	1934	1935*
% smut grown and harvested repeatedly on *nuda*					
15	15	7	10	0	0
Smut grown on potato 1930–2 then tested on *nuda*					
—	—	9	7	0	0

* In 1935 it was necessary to use 1933 spores owing to failure on *nuda* in 1934.

The implication from such an experiment is that the power to produce slight infection on *nuda* is a fairly stable character in collection C_4, since the infection during the years 1927–33 ranged from 7 to 28%, and no sudden change resulted from screening. The results with monospore lines were entirely at variance with such a conclusion. The seven monospore lines isolated in 1933 and tested on both Potato and *nuda* fall into two distinct groups (Table IV). $C_{4/2}$, $C_{4/3}$ and $C_{4/5}$ gave full infection on both hosts, while $C_{4/1}$, $C_{4/8}$, $C_{4/12}$, and $C_{4/13}$ infected only Potato. The data have been examined, and it is impossible to explain them by the presence of chance impurities in either host or fungus. It is evident that the infection of *nuda* by collection C_4 is due to the presence of the potato-*nuda* type which was revealed when monospore isolations were studied but, even allowing for hybridization between the two types, it seems odd that screening on *nuda* failed to change the degree of infection on that host. Further light may be thrown on the problem by a study of monospore isolations from those lines which infected both the hosts and from the behaviour of the two types in mixture.

[1] These were regarded as duplicates and only C_4 was maintained in cultivation after 1927.

A summary of the behaviour of all the spore collections is given in Table VI which shows the salient features of the monospore lines at present in cultivation.

Table VI

Behaviour of six collections of oat smut over a 10-year period of propagation involving both screening and the isolation of monospore lines

Ustilago Avenae

	L_2	L_{11}	L_{12}
1927	Potato Abundance *nuda* *strigosa*	*strigosa* *brevis*	Potato
1937	Lost by screening the capacity to infect *strigosa*	No change	No change

Ustilago Kolleri

	C_1	C_2		C_4	
1927	*strigosa* *brevis*	Potato *nuda* *strigosa*		Potato *nuda* (slight)	
1937	No change	No change	Lost capacity to attack *strigosa*	Potato *nuda* (100 %)	Potato

(2) *Cultural characteristics of monospore lines*

Dickinson (1928) and Holton (1932) studied the cultural characteristics of monosporidial lines and showed that they segregate in a regular manner on the germination of the chlamydospores. A colony derived from a single chlamydospore may, therefore, represent not a single phenotype, but a population of haploid mycelia of different types. It might be expected that the characteristics of the different types would mask each other and that the features of a monospore colony would be more difficult to define and less constant in subculture than those of a monosporidial colony. It would not be surprising if false sectoring were a common phenomenon arising from the predominating growth of a particular type at one side of the colony. In point of fact sectoring was absent in the monospore colonies, and they showed rather well defined characteristics which reappeared with somewhat unexpected regularity in subsequent transfers. That these features were a composite effect and that segregation for cultural characteristics did occur in the promycelium was shown by a comparison of monospore and monosporidial cultures

Table VII

The chief cultural characteristics of six spore collections. Description based in each case on five monospore lines. Age of colonies 45 *days*

Ref. to collection	Centre of colony: Colour	Centre of colony: Type	Margin of colony: Colour	Margin of colony: Type	Range in diameter in mm.
C_1	Brown	Slightly raised with central depression. Glossy	Greyish brown	Regularly circular. Inconspicuously zonate	43–45
C_2	Yellow changing to brown with age	Raised, small, with an eccentric depression	Greyish white	Dense white median band. Peripheral radial striations	29–33
C_4	Buff to brown	Raised with a regular "double centre"	White	Sharply zonate with radiating hyphae at periphery	36–43
L_2	Brown	Slightly raised, small, glossy	Greyish brown	Closely zonate, some cultures with well defined lighter bands. Radial striations	38–46
L_{11}	White	Large, convoluted	Opaque milky white	Zonate with pronounced bands. Lobate margins	36–43
L_{12}	Brownish	Small, slightly convoluted. Glossy	Greyish brown	Indistinct zonation. Dull coloured broader bands in some cultures	42–46

from one collection (L_2). There is no doubt that monosporidial isolates are essential for a precise study of cultural features in the smut fungi, but the following observations concerning the growth on an artificial medium of five monospore lines obtained from each of the spore collections under discussion are not without interest. The salient features of each collection are summarized in Table VII. Familiarity with the cultures made it possible to recognize individual peculiarities such as width of zonation which reappeared consistently in parallel transfers, but they defy exact description and it would serve no useful purpose to dwell on them here. The facts which call for emphasis are as follows: (1) The absence of any distinguishing cultural characteristics between the taxonomic species[1] *Ustilago Avenae* and *U. Kolleri*. A similar result was recorded by Rodenhiser (1928). (2) The absence of any correlation between pathogenicity and growth in culture. For example, collections L_{11} and C_1 possess quite different cultural characteristics and monospore

[1] These might with justice be regarded as echinulate and smooth spored varieties of the same species.

line $C_{2/5}$ did not show a closer resemblance to $C_{4/2}$ than did the other C_2 lines (see Table IV for pathogenicity). (3) The extraordinary uniformity of monospore cultures within the collection L_{11}. The white convoluted centre and opaque milky white margin made it possible for any observer to pick out any one of the five monospore lines of L_{11} and the distinction was not lost in old cultures or in subcultures. Moreover, when this collection was selected for a study of monosporidial lines, they showed, in contrast with those of L_2, almost perfect uniformity, indicating the complete absence of segregation in any characteristic which could readily be defined (Pl. XX).

(3) *The stability of resistance to smut in oats*

The resistance of an oat variety to a particular collection of smut spores is measured normally by the percentage of plants which carry smutted panicles when shelled grains are heavily dusted with spores and sown under optimum conditions for infection. The degree of infection in a highly susceptible variety depends largely upon the environment of the grain at the time of germination, a low moisture content favouring infection (Sampson, 1929). Little information is available concerning the later effect of environment on the development of smut. This is a matter of particular interest, as so-called resistant varieties may carry mycelium in their basal tissues even until maturity, without the production of smutted panicles. Can this resistance be changed by any method, or do host and parasite make the same response to an altered environment?

Experiments designed to answer this question have been made at Aberystwyth and are summarized below.

In 1925, starting with somewhat poor soil, artificial manures were used to improve the growth of the crop. Phosphates in particular gave very high increases in yield, but failed to influence the percentage of smutted plants.

For two seasons (1927–8) pot experiments were conducted in which growth was increased or reduced by varying both the moisture of the soil and the nitrogen supply. Tillering in particular varied widely in the different series giving a range per plant of 1·8–4·4 in *nuda* and 1·9–6·0 in Record, but there was no correlation between this or any other aspect of growth and the development of smut.

In 1933–4 an extensive experiment was set up using shelled grains of *strigosa* contaminated with spores of collection C_4 to ascertain if smutted panicles could be induced by cutting back the plants after different

intervals of time. Germination was carried out under conditions which are known to allow the smut to enter the plant (Sampson, 1933). In no case did the removal of the first-formed tillers influence the degree of visible infection; a similar result was obtained by Western in other experiments.

Additional attempts to break down the resistance of a variety were made in 1934, using another collection (L_2) which invades var. *strigosa* but fails to produce spores. Hypodermic inoculation of the growing points of young oat seedlings was carried out with (1) a suspension of spores, and (2) a suspension of two monosporidial lines of opposite sex in distilled water. In each case the result was negative.

In another experiment grains dusted with the spores of L_2 were grown for 3 days under optimum conditions for infection and then kept at a low temperature (2 and 5° C.) for 4 weeks. Growth was checked but, subsequently, the plants matured normally producing healthy panicles.

It is concluded that it is not easy to break down the resistance of oats to smut though, admittedly, there are other methods such as the use of narcotics and high temperatures which have not been tried. Little experimental work has been done on the possibility of changing the relationship in the direction of increased resistance.

IV. Discussion

Although cytological evidence is still scanty, results have been obtained during the past 20 years which establish on a firmer basis the supposition that segregation on Mendelian lines takes place in the fungi.

So far as we know at present, the oat smuts are non-pathogenic in the haploid condition, but the invading parasitic mycelium may be derived either from a pair of fused sporidia or other functional gametes, or from the promycelium which is the immediate result of germination in the chlamydospore. Western (1937) obtained evidence which suggests that the latter method is the one which happens most frequently under natural conditions, and this is a point which bears directly on the behaviour of different races in mixture. To take an extreme case, it seems as if it would be possible theoretically for the heterozygous condition to persist through a number of chlamydospore generations, and it is probably this which limits the efficiency of screening experiments as a means of obtaining races genetically pure for pathogenicity characters.

The persistency of the heterozygous condition seems to be the most likely explanation for some of the facts presented in this paper. From

collection C_2 two types were obtained (in 1933) in the monospore lines; type (*a*) behaved like the parent collection in that it infected Potato, *nuda* and *strigosa*, while type (*b*) infected only the first two hosts. In 1931 and in 1932 collection C_2 was grown on *strigosa*, and one might have expected that this would have screened out type (*b*) if it existed as such in the original collection. The implication is that type (*a*) probably represents the 1927 collection, and that it is segregating for the capacity to infect *strigosa*. It is not yet known in what manner or how frequently this segregation takes place, since data on later generations are still incomplete.

Collection C_4 also yielded two types, but in this case both differed from the parent collection, the one infecting *nuda* more completely and the other giving no smutted panicles on *nuda*. The consistently positive but low infection obtained with the parent collection for a number of years must, in some way, be related to the presence of these types in the 1927 collection, yet it is obvious they could not have been there as a mechanical mixture. This would seem to be another example of the persistency of the heterozygous condition through several generations of chlamydospores produced in the host plant, but data are not yet available for a satisfactory explanation of the behaviour of collection C_4 and its component types. Nicolaisen (1934), working with a number of different spore collections, concluded that they represented a number of distinct biotypes which were heterozygotic for both cultural and pathogenic characters.

Another aspect of the results which is worthy of emphasis is the tendency to obtain a clear-cut distinction between susceptibility and resistance. The authors have not yet found a pure-breeding line which has the capacity of producing consistently a low infection upon a certain oat variety. This is in agreement with the sharply delimited grades of resistance already described in histological studies (Sampson, 1933; Western, 1936).

V. SUMMARY

1. An attempt has been made to trace the history of six spore collections of the oat smuts during a 10-year period of experimental work and to compare them in regard to stability.[1]

2. Evidence of change was found in three collections and the modifications, with one exception, were in a negative direction.

3. At an early stage in the work, collection L_2 lost by screening the capacity to attack *strigosa*.

[1] See footnote p. 493.

4. By monospore isolations, collection C_2 was resolved into two types, one of which had a narrower range of infection than the parent collection.

5. Collection C_4 also yielded two types, but both differed from the original collection. One was more pathogenic and the other less pathogenic than the collection as a whole.

6. Reasons are given for the view that the changes were due to heterozygous types in the collections.

7. No changes were detected in three collections.

8. One collection (L_{11}) was outstanding in the uniformity of monospore and monosporidial lines in culture.

9. Experiments designed to break down the resistance to smut in certain selected oat varieties met with no success.

VI. Acknowledgements

The authors desire to express their gratitude to Dr S. Dickinson for advice in isolation technique, and to Mr E. T. Jones, M.Sc. for his helpful interest in the work. Sincere thanks are given to Prof. E. C. Stakman for the kindness and hospitality shown to the junior author in the year 1935–6. Grateful acknowledgement is made of the facilities granted by Prof. R. G. Stapledon, C.B.E., M.A., and of the careful supervision of experiments by Mr J. W. Watkins.

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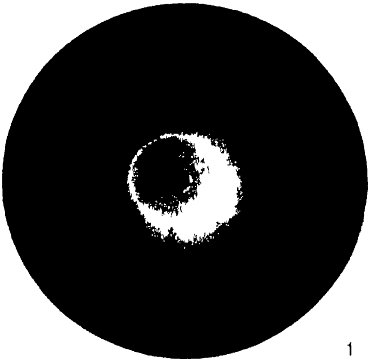

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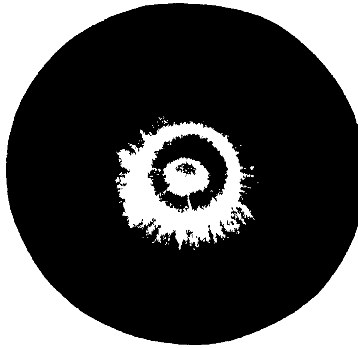

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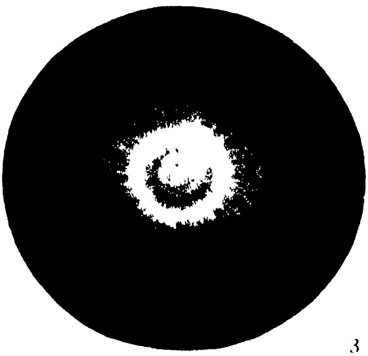

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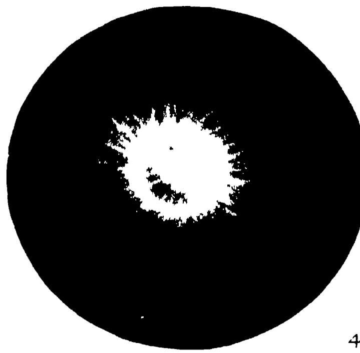

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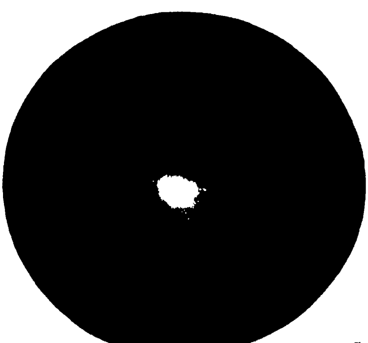

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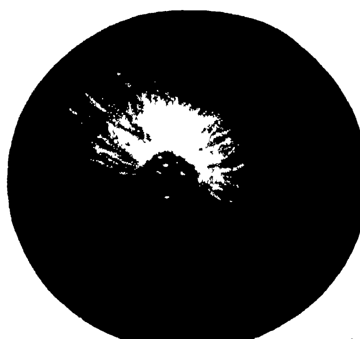

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EXPLANATION OF PLATE XX

The authors are indebted to Mr D. Walters Davies, B.Sc., for assistance in photographing the cultures.

Figs. 1–4. Four monosporidial lines from the four segments of the promycelium of a single chlamydospore of L_{11}, showing no evidence of segregation for cultural characteristics.

Figs. 5–6. Two contrasting monosporidial lines from a single chlamydospore of L_2.

Colonies 45 days old on artificial medium (see p. 501).

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A STUDY OF CROWN RUST, *PUCCINIA CORONATA* CORDA, IN GREAT BRITAIN

II. THE AECIDIAL HOSTS OF *P. CORONATA*[1]

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(With Plate XXI and 1 Text-figure)

CONTENTS

Introduction

In a previous paper (Brown, 1937), it was reported that the following varieties of *Puccinia coronata* Corda could be differentiated in Great Britain by their pathogenicity in the uredospore stage:

(1) var. *alopecuri* infecting *Alopecurus pratensis*,

(2) var. *arrhenatheri* infecting *Arrhenatherum avenaceum*,

(3) var. *avenae* infecting *Avena* spp.,

(4) var. *calamagrostidis* infecting *Calamagrostis lanceolata* and *Phalaris arundinacea*,

(5) var. *festucae* infecting *Festuca elatior*,

(6) var. *holci* infecting *Holcus lanatus*,

(7) var. *lolii* infecting *Lolium perenne*.

The present paper records experiments to determine the relationship between these varieties and the two species of *Rhamnus*, *R. Frangula* and *R. cathartica*, which are the aecidial hosts of the fungus in this country.

[1] This work was carried out from 1932 to 1934 at the Cambridge University Botany School and from 1934 to 1935 at the Dominion Rust Research Laboratory, Winnipeg, Canada.

Klebahn (1892–1912), Eriksson (1894–1909) and Mühlethaler (1910, 1911) found that the varieties of crown rust obtained in Europe had their aecidial stage either on *R. Frangula* or on *R. cathartica*, but not on both. For this reason Klebahn (1892) divided the original species *Puccinia coronata* Corda into two, retaining the name *P. coronata* for those varieties with their aecidial stage on *Rhamnus Frangula*, and giving the name *Puccinia coronifera* to those with their aecidial stage on *Rhamnus cathartica*. The varieties *calamagrostidis* and *agrostidis* were included in the former species and the varieties *avenae*, *alopecuri*, *festucae*, *lolii*, *glyceriae* and *epigaei* in the latter. As rust from *Holcus* spp. and *Agropyron repens* appeared sometimes to infect *Rhamnus Frangula* and sometimes *R. cathartica*, it was considered that these grasses were infected by varieties of rust belonging to both species.

Treboux (1912), working in Russia, found that the supposedly "*coronata*" hosts, *Calamagrostis lanceolata*, *Phalaris arundinacea* and *Agrostis stolonifera*, were sometimes infected by aecidiospores from *Rhamnus cathartica*, and at a later date (1914) he found that the "coronifera" host, oats, could be slightly infected with aecidiospores from *R. Frangula*. He considered that the distinction between the two species was not as clearly cut as had been supposed by Klebahn and Eriksson.

A similar conclusion was reached by Melhus *et al.* (1922) and Dietz (1926*a*), who investigated the aecidial hosts of *Puccinia coronata* in the United States. They inoculated a number of *Rhamnus* species with sporidia from teleutospores on oats, *Calamagrostis canadensis* and *Festuca elatior*, and found no marked difference in the alternate host range of rust from these grasses. *Rhamnus cathartica* and other closely related species could be infected heavily by teleutospores from all three hosts, but the spores on oats and *Calamagrostis canadensis* also caused slight infection of *Rhamnus Frangula*. It appeared, therefore, that Klebahn's division of *Puccinia coronata* Corda into two species did not hold for American material.

In 1931, four pathogenic types of crown rust were distinguished in Canada by Fraser & Ledingham (1933). The aecidial stages of these forms occurred on *Rhamnus cathartica*, *R. alnifolia*, *Lepargyrea canadensis* and *Eleagnus commutatus* respectively, and slight morphological differences were observed between them. They were described as varieties of *Puccinia coronata* Corda and not as separate species.

Little work has been done in England on the aecidial hosts of *P. coronata*. Plowright (1889) obtained aecidia on *Rhamnus Frangula* by inoculation with teleutospores on *Dactylis glomerata* and *Festuca sylvatica*

but failed to infect this host with teleutospores on *Lolium perenne.* Hanes (1936) inoculated oats, wheat, barley, rye, *Lolium perenne,* and *L. italicum* with aecidiospores collected in the field on *Rhamnus Frangula* and *R. cathartica.* Only hypersensitive flecks were produced, although both oats and *Lolium perenne* were sometimes heavily infected in the neighbourhood in which the experiments were carried out.

Mr T. G. Jennings (unpublished work carried out at the Cambridge Botany School in 1931–2) inoculated *Rhamnus Frangula* and *R. cathartica* with teleutospores on *Calamagrostis lanceolata, Lolium perenne* and oats. The spores on *Calamagrostis lanceolata* infected only *Rhamnus Frangula* and those on oats and *Lolium perenne* only *Rhamnus cathartica.* He also inoculated a number of grasses with aecidiospores collected in the field on the two species of *Rhamnus,* and found that oats, *Dactylis glomerata, Holcus lanatus, Alopecurus pratensis* and *Lolium perenne* were infected only by aecidiospores from *Rhamnus cathartica,* while *Calamagrostis lanceolata* and *Phalaris arundinacea* were infected by aecidiospores from both species. These results appeared to be contradictory, since the experiments with teleutospores indicated that the varieties of *Puccinia coronata* could be clearly divided into two groups according to their aecidial host, in agreement with the results of Klebahn and Eriksson, while the experiments with aecidiospores seemed to show that they could not be differentiated in this way, in agreement with the results of Treboux and Melhus *et al.*

The experiments described in the present paper were carried out on the same lines as those of Jennings. Seedling plants of *Rhamnus Frangula* and *R. cathartica* were inoculated with sporidia from germinating teleutospores on a number of grasses, and the grasses were in turn inoculated with aecidiospores collected in the field on the two species of *Rhamnus.*

Inoculations with sporidia

(a) *Source of host plants*

Seedlings of *Rhamnus Frangula,* 1–2 years old, were collected in the autumn at Wicken Fen, near Cambridge, and kept in a cold frame until required for experiment in the following spring. Seedlings of *R. cathartica* were grown from seed in a greenhouse, as they were difficult to find in the field. In the few experiments in which the Canadian species, *R. alnifolia,* was used, young plants were collected in a wood near the Dominion Rust Research Laboratory, Winnipeg, and were transferred to pots in a greenhouse. Cut shoots of this species were also employed as they survived long enough in water for the production of aecidia.

(b) *Source of teleutospores*

Teleutospores were produced in the greenhouse at Cambridge by the varieties *calamagrostidis*, *alopecuri*, *arrhenatheri*, *avenae*, *lolii* and *holci*. They were formed mainly in the summer and early autumn and, as they would not germinate without being frozen, the plants bearing them were placed out of doors during the winter. In this way a good supply of germinating spores was obtained in the following spring.

An attempt was made to germinate teleutospores formed on oats without previous overwintering, using the technique devised by Johnson (1931) in experiments with the teleutospores of *Puccinia graminis tritici*. Pieces of straw bearing the spores were frozen in blocks of ice at -5° C. for a month. They were then thawed, soaked in tap water for 24 hr., placed on filter paper in a Petri dish and subjected to alternate periods of wetting and drying, the moist and dry periods each consisting of about 24 hr. In four out of six samples slight germination was obtained, but only in one was it sufficient to cause infection of plants of *Rhamnus cathartica*. The time elapsing between the placing of the teleutospores in the refrigerator and the appearance of aecidia on the *Rhamnus* was 71 days, a period considerably shorter than would have elapsed under natural conditions between the formation of teleutospores and the production of aecidia.

Teleutospores were collected in the field on *Calamagrostis lanceolata*, *Phalaris arundinacea*, *Arrhenatherum avenaceum*, *Avena sativa*, *A. strigosa*, *Holcus lanatus* and *Lolium perenne*. They were allowed to overwinter out of doors and germinated well in the following spring.

(c) *Method of inoculation*

Grass leaves bearing germinable teleutospores were soaked in tap water for several hours and then tied among the young leaves of the plant to be inoculated. Both plant and teleutospore material were sprayed thoroughly with tap water from an atomizer, and placed under a bell-jar lined with damp blotting paper for 48 hr. Care was taken to keep the blotting paper moist throughout this period and the plants were sprayed at intervals to maintain a film of moisture on the surface of the leaves.

In some experiments, the plant to be inoculated was covered by a glass lamp chimney and the teleutospore material was placed on damp filter-paper in a watch-glass or Petri dish inverted over the top of this chimney. The sporidia were thus shot off their sterigmata on to the leaves of the plant. Spraying and incubation were carried out as before. This method, used by Newton & Johnson (1932) for the inoculation of barberry plants with sporidia of *Puccinia graminis*, was useful for producing scattered infection spots suitable for hybridization experiments, but, for testing the susceptibility of a *Rhamnus* species to an individual variety of rust, the first method proved more satisfactory.

After inoculation, the plants were placed out of doors enclosed in cages to exclude insects. These cages consisted of cubical wooden frames the sides of which were covered with two thicknesses of fine butter muslin, and the tops with a sheet of glass. They were effective in keeping out the majority of insects, though occasional specimens of thrips and aphides were found inside them. Each cage held nine 5½ in. pots, and only plants inoculated with the same variety of rust were placed together in a cage.

In each experiment, one plant of the *Rhamnus* species expected to be susceptible to the rust variety used was inoculated at the same time as two or three plants of the

species expected to be resistant. This was done to ensure that there was adequate opportunity for the supposedly resistant species to become infected. Experiments with an individual variety were repeated until definite conclusions could be drawn as to its power of infecting the *Rhamnus* species.

The experiments were carried out in May and June of the years 1933, 1934 and 1935.

(*d*) *Results.* The first sign of infection on the inoculated plants was the appearance of minute, yellow, slightly raised spots on the young leaves. These infection spots or pustules appeared 5–10 days after inoculation and gradually increased in size, frequently becoming confluent where they lay close together. About 3 days after their appearance, spermogonia could be discerned in them which soon began to secrete nectar.

According to Allen (1932) *Puccinia coronata* is heterothallic. A monosporidial infection will not produce aecidia unless it is sufficiently near an infection of opposite strain for the two mycelia to coalesce, or unless it is fertilized by spermatia of opposite strain. Since insects were excluded from the experimental plants, nectar from one pustule was conveyed to another on the point of a sterilized needle, each pustule receiving nectar from several others in order to ensure that the right strain was present for fertilization. When the pustules were close together and showed some coalescence, aecidia were produced without this mixing of the nectar.

Aecidia began to appear 6–10 days after fertilization. They developed on the under surface of the pustules which, by this time, had increased considerably in size, and were associated with a thickening of the lamina owing to local hypertrophy. When infection was particularly heavy aecidia were also formed on the young twigs, which became much swollen and distorted.

Table I shows the results obtained when *Rhamnus Frangula* and *R. cathartica* were inoculated with teleutospores of the different varieties of *Puccinia coronata*. These varieties can be divided into two groups according to their aecidial host: var. *calamagrostidis* produces aecidia only on *Rhamnus Frangula* while vars. *alopecuri*, *holci*, *arrhenatheri*, *lolii*, *festucae* and *avenae* produce aecidia only on *R. cathartica*.

The percentage of infection obtained with the varieties *lolii*, *festucae*, and *avenae* was rather low, chiefly owing to poor germination of the teleutospores. Infections occurred, however, always on *R. cathartica* and never on *R. Frangula*, although numerous plants of the latter were inoculated. It was concluded that *R. cathartica* was the aecidial host of these varieties, a conclusion which was confirmed, in the case of the

var. *lolii* by the results of inoculations with aecidiospores to be described later.

Table I

Infection of Rhamnus Frangula *and* R. cathartica *with teleutospores of* Puccinia coronata Cda

			R. Frangula			*R. cathartica*		
Variety of *P. coronata*	Host bearing teleuto-spores	Origin of teleuto-spores	No. of plants inocu-lated	No. of plants bearing spermo-gonia and aecidia	No. of plants bearing spermo-gonia only	No. of plants inocu-lated	No. of plants bearing aecidia and sper-mogonia	No. of plants bearing spermo-gonia only
Calamagros-tidis	*Calamagrostis lanceolata*	Greenhouse and field	16	9	1	39	0	0
„	*Phalaris arundinacea*	Greenhouse and field	22	15	0	48	0	2
Alopecuri	*Alopecurus pratensis*	Greenhouse	16	0	0	8	6	1
Holci	*Holcus lanatus*	Greenhouse and field	19	0	0	10	3	3
Arrhenatheri	*Arrhenatherum avenaceum*	Greenhouse and field	12	0	0	6	2	2
Lolii	*Lolium perenne*	Greenhouse and field	53	0	0	29	2	8
Festucae	*Festuca elatior*	Field	14	0	0	7	1	1
Avenae	*Avena sativa*	Greenhouse	6	0	0	6	1	0
	Avena strigosa	Field	33	0	0	11	0	3

Only one experiment gave evidence of the infection of both species of *Rhamnus* by teleutospores from a single grass species. In this two seedlings of *R. Frangula* and four of *R. cathartica* were inoculated with teleutospores collected in the field on *Phalaris arundinacea*. There was copious production of spermogonia and aecidia on *Rhamnus Frangula* but a few spermogonia were also produced on two of the plants of *R. cathartica*. These spermogonia were small and abortive, secreting very little nectar, and there was no sign of aecidial development. By the time a heavy crop of aecidia had appeared on the leaves of *R. Frangula*, the spermogonia on *R. cathartica* were much blackened and shrivelled (Pl. XXI, fig. 1).

Microtome sections were cut of the infected leaves of both species, which were fixed 13 days after inoculation in formalin-chrome-acetic solution and stained with iron-alum and haematoxylin, or diamant fuchsin and light green. The leaves of *R. Frangula* showed abundant production of mycelium, well developed spermogonia with numerous spermatia, aecidial initials on the under-surface of the leaf and considerable hypertrophy of the host cells, which were never necrotic

(Pl. XXI, fig. 2). In *R. cathartica* (Pl. XXI, fig. 3) the mycelium was poorly developed and the spermogonia small and abortive, producing few spermatia. There was no sign of aecidial initials and little hypertrophy of the host. Some of the host cells, particularly those of the upper epidermis, were collapsed and necrotic.

Hand sections, cut at the close of the experiment, 28 days after inoculation, showed well developed aecidia on *R. Frangula* and a copious mycelium ramifying through the mesophyll, which was hypertrophied but not necrotic. On *R. cathartica* the infection still showed spermogonia only; the mycelium was less well developed than in *R. Frangula* and the infection spot was surrounded by a zone of necrotic cells stretching from the upper to the lower epidermis.

Ruttle & Fraser (1927), investigating the cytology of the uredospore stage of *Puccinia coronata*, found that on the susceptible oat variety, Banner, the mycelium developed copiously, with no necrosis of the host cells, but on the partially resistant variety Cowra only a scanty mycelium appeared, and there was considerable necrosis. Hanes (1936) also found that when aecidio- and uredospores of *P. coronata* were inoculated on to resistant hosts there was poor development of mycelium and some necrosis. The type of infection obtained on *Rhamnus cathartica* therefore resembles, in many respects, that produced by uredospores on a semi-resistant host, and it seems probable that, although normally no sign of infection is produced on *R. cathartica* by var. *calamagrostidis*, occasionally the immunity may be partially broken down and development of the fungus may proceed as far as the formation of abortive spermogonia. The spread of the fungus appears, however, to be limited by the necrosis of the surrounding host cells in the same way as the spread of the uredospore stage is limited in semi-resistant hosts.

Instances of the infection of both *R. Frangula* and *R. cathartica* by teleutospores of *Puccinia coronata* from the same species of grass have been recorded in North America by Melhus *et al.* (1922), Dietz (1926*a*) and Fraser & Ledingham (1933). These workers have found that teleutospores on oats cause heavy production of aecidia on *Rhamnus cathartica* but may, also, form a few scattered spermogonia on *R. Frangula*. Because of these indecisive results, and because of the large number of *Rhamnus* and related species which may be infected by crown rust in the United States and Canada, they consider that Klebahn's division of the rust into two species does not hold for American material.

Except for the single instance described above, the varieties of *Puccinia coronata* occurring in Great Britain appear to infect either

Rhamnus Frangula or *R. cathartica* but not both, and the results thus agree with those of the European workers Klebahn, Eriksson and Mühlethaler. Whether this difference in the aecidial host is sufficient, in the light of our present knowledge of the rust fungi, to justify the division of *Puccinia coronata* Corda into two species, will be discussed later.

A few experiments were carried out with teleutospores of *P. coronata* collected in Canada on *Calamagrostis canadensis*. These teleutospores were inoculated on to *Rhamnus Frangula*, *R. cathartica* and also *R. alnifolia*, the North American species which had previously been shown by Melhus *et al.* (1922), Dietz (1926*a*) and Fraser & Ledingham (1933) to be the aecidial host of crown rust on this grass. Aecidia were obtained only on *R. alnifolia*, thus indicating that the variety of crown rust infecting *Calamagrostis* in Canada differs from that infecting the same genus in Great Britain in its aecidial, as well as in some of its uredinial hosts (Brown, 1937).

INOCULATIONS WITH AECIDIOSPORES

(*a*) *Source of aecidiospores*

The aecidiospores used in these experiments were collected in the field on *Rhamnus Frangula* and *R. cathartica* during the summers of 1933 and 1934. The collections on *R. Frangula* were made at Wicken Fen, this being the only place in the neighbourhood where infected bushes were found in any abundance. The collections on *R. cathartica* were made at Wicken Fen, at the Cambridge University Farm and in the University Botanic Garden.

(*b*) *Method of inoculation*

The aecidiospores were inoculated on to the grasses and oat varieties used in the experiments with uredospores (Brown, 1937), and listed in Tables II and III. The seedling grasses were inoculated when five or six leaves had developed, and the oats were inoculated on the first leaf when only this had emerged. The method of inoculation was as follows: the aecidiospores were scraped off a number of infected leaves into a sterilized Petri dish and were thoroughly mixed together to prevent discrepancies in the results due to the occurrence of only one variety of rust in a single aecidium or a single aecidial pustule. They were then transferred on a sterile scalpel to the upper surface of the leaves to be inoculated, which were marked with Indian ink for identification. The plants were then sprayed thoroughly with tap water by means of an atomizer and were incubated under bell jars standing over water for 48 hr. After the incubation period, they were placed on the greenhouse bench and covered with cellophane cages. As a rule, each collection of aecidiospores was inoculated on to the complete range of grasses and oat varieties, and the inoculations with spores from the two species of *Rhamnus* were always made on the same day, so that direct comparisons could be made of their infective capacities.

Uredospore pustules began to appear about 10 days after inoculation, and when these were fully developed, about 5 days later, notes were taken of the number of plants infected and the type of infection produced. The infection types were classified according to the scheme drawn up by Stakman & Levine (1922) for the infection types produced on wheat by *Puccinia graminis tritici,* and were divided into five classes, designated by the symbols 0 to 4, as described below:

Table II

Results obtained on inoculation of grasses with aecidiospores collected in the field on Rhamnus Frangula *and* R. cathartica.

Summers 1933 *and* 1934

Species inoculated	Aecidia on *R. Frangula*: Total no. of plants: Inoculated	Infected	Infection type	Aecidia on *R. cathartica*: Total no. of plants: Inoculated	Infected	Infection type	
Lolium perenne	34	0	0	78	43	0, 3, 4	Group I
Holcus lanatus	34	0	0	69	31	0, 3, 4	
Alopecurus pratensis	27	0	0	64	23	0, 2, 3, 4	
Arrhenatherum avenaceum	34	0	0	58	26	0, 4	
Festuca elatior	30	0	0	48	16	0, 3, 4	
Dactylis glomerata	34	1	0, 2	65	7	0, 1, 2, 3	Group II
Calamagrostis lanceolata	44	42	4	55	25	0, 1, 2, 3, 4	
Phalaris arundinacea	34	32	3, 4	63	2	0, 1, 2	
Agropyron repens	30	0	0	45	0	0	Group III
Bromus sterilis	29	0	0	54	0	0	
Agrostis palustris	16	0	0	34	0	0	

Host reaction	Symbol	Infection type
Highly resistant	0	No uredospore pustules developed; necrotic or chlorotic flecks usually present.
Very resistant	1	Pustules minute and isolated; usually accompanied by pronounced necrosis; necrotic spots often produced without development of pustules.
Moderately resistant	2	Pustules small to medium in size; surrounded by necrotic or markedly chlorotic areas; necrotic spots rarely without pustules.
Moderately susceptible	3	Pustules fairly abundant; medium sized; no necrosis, but chlorosis usually present.
Very susceptible	4	Pustules large and abundant; often confluent and showing copious production of uredospores; no necrosis but occasional slight chlorosis.

(The *X* type of infection in which all five types are found on the same leaf did not occur in these experiments.)

The experiments were carried out in June and July 1933 and 1934 in an unheated greenhouse whose average daily temperature ranged from 58 to 79° F.

(*c*) *Results*

(i) *Inoculation of grass species.* The results obtained on the inoculation of the grass species are shown in Table II. It was found that individual plants of the same species differed very much in their degree of infection

even when inoculated with aecidiospores from a single sample. This was probably due partly to the occurrence of a number of different varieties of rust in the aecidial inoculum and partly to variations in the constitution of the host plants.

In spite of these variations, the grasses could be clearly divided into three groups:

I. Grasses which were infected by aecidiospores from *Rhamnus cathartica*, but not by those from *R. Frangula*.

II. Grasses which were infected by aecidiospores from both species of *Rhamnus*.

III. Grasses which were not infected by aecidiospores from either species.

The grasses falling into Group I are *Lolium perenne*, *Holcus lanatus*, *Alopecurus pratensis*, *Arrhenatherum avenaceum* and *Festuca elatior*. They showed infections of the 3 and 4 types on 33–55% of the plants inoculated with aecidiospores from *Rhamnus cathartica*, but only flecking or no sign of infection on the plants inoculated with aecidiospores from *R. Frangula*.

In order to identify the varieties of rust which caused the infections, uredospores from each grass were inoculated on to as many as possible of the series of grass species used for the differentiation of varieties in the experiments with uredospores (Brown, 1937). It was shown that the infections on *Lolium perenne* and *Festuca elatior* were due to the variety *lolii*, those on *Holcus lanatus* to the variety *holci*, those on *Arrhenatherum avenaceum* to the variety *arrhenatheri*, and those on *Alopecurus pratensis* to the two varieties *alopecuri* and *lolii*. This last result is in agreement with the findings in the experiments with uredospores, in which the variety *lolii* was not confined to *Lolium perenne* but was capable of causing moderate infection on certain other grasses, including *Alopecurus pratensis*. The variety *festucae* did not appear to be present in this aecidial inoculum although it was observed in the neighbourhood in the uredospore stage.

The aecidiospores obtained from *Rhamnus cathartica* thus belonged to the varieties *lolii*, *holci*, *arrhenatheri*, and *alopecuri*. This is in agreement with the results of the experiments with teleutospores, in which the sporidia of these varieties infected *R. cathartica* but not *R. Frangula*.

The grasses of Group II, which were infected by aecidospores from both species of *Rhamnus*, were *Calamagrostis lanceolata*, *Phalaris arundinacea* and *Dactylis glomerata*. The first two were infected more heavily

by the aecidiospores from *Rhamnus Frangula* and the last by those from *R. cathartica*.

In the experiments with teleutospores; rust on *Calamagrostis lanceolata* and *Phalaris arundinacea* gave rise to aecidia only on *Rhamnus Frangula* and did not infect *R. cathartica*, so that the results of the two sets of experiments seemed to be contradictory. The apparent discrepancy, however, was explained when the varieties responsible for the infections were identified as far as was possible with the limited amount of inoculum available.

The heavy infections obtained on *Calamagrostis lanceolata* and *Phalaris arundinacea* when inoculated with aecidiospores from *Rhamnus Frangula* were shown to belong to the variety *calamagrostidis*, but the infections produced on these grasses by aecidiospores from *R. cathartica* were so scanty that it was impossible to inoculate more than a small number of the differential hosts. A few tests were, however, carried out with uredospores from *Calamagrostis lanceolata*, and the results are shown in Table III. In the circumstances it was necessary in planning the tests, to decide which varieties of rust were most likely to be present, and to choose for inoculation those grasses which would differentiate them most clearly. In the experiments with uredospores (Brown, 1937) it was found that *Calamagrostis lanceolata* was infected most heavily by var. *calamagrostidis*, but was also somewhat susceptible to vars. *lolii*, *alopecuri*, *festucae* and *avenae*. Vars. *lolii* and *alopecuri* were known to be present in the aecidial inoculum so that it seemed likely that they, or var. *calamagrostidis*, were responsible for the infection. Of these three varieties: var. *alopecuri* causes heavy infection on *Alopecurus pratensis*, light infection on *Calamagrostis lanceolata* and no infection on *Lolium perenne*; var. *lolii* infects *Lolium perenne* heavily and *Alopecurus* and *Calamagrostis* with infection types ranging from 1 to 3; while var. *calamagrostidis* only infects *Calamagrostis*. The varieties could therefore be differentiated by means of these three grasses, which were accordingly used in the experiments. Sufficient inoculum was not however available for each of them to be used in every test.

Table III shows that in tests I and II *Lolium perenne* was heavily infected and the variety present was therefore var. *lolii*; moreover, the lack of infection on *Calamagrostis lanceolata* confirmed the absence of var. *calamagrostidis*. In test III, only *Lolium perenne* was inoculated, and as it was not infected, it was impossible to draw any conclusions as to the nature of the variety, except that it was probably not var. *lolii*. In test IV, *Alopecurus pratensis* was infected but not *Lolium perenne*, so

that the infection was evidently due to var. *alopecuri*. In test V only *Calamagrostis lanceolata* was infected, but the pustules were very small and some were surrounded with necrotic areas, which are not obtained when the grass is infected with var. *calamagrostidis*. The spore production was so scanty that it was impossible to maintain the rust for further experiment, and no conclusions could be reached as to the variety responsible for the infection.

Table III

Infection types produced by first generation of uredospores on Calamagrostis lanceolata *inoculated with aecidiospores from* Rhamnus cathartica. (*Tests carried out June-July* 1934)

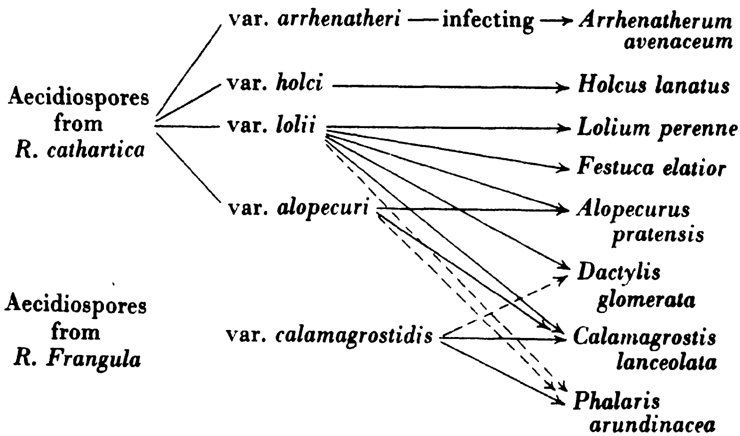
Test no.	Source of aecidiospores	Infection type produced on *C. lanceolata*	Grasses inoculated with uredospores from *C. lanceolata*	No. of plants Inoculated	Infected	Infection type	Variety causing infection
I	*R. cathartica* University Farm	2	*Lolium perenne*	2	2	4	*lolii*
II	*R. cathartica* University Farm	2	*Lolium perenne*	1	1	4	*lolii*
			Calamagrostis lanceolata	1	0	0	
III	*R. cathartica* Botanic Garden	3	*Lolium perenne*	2	0	0	?
IV	*R. cathartica* Botanic Garden	4	*Lolium perenne*	2	0	0	*alopecuri*
			Alopecurus pratensis	2	2	4	
V	*R. cathartica* Wicken Fen	3	*Lolium perenne*	1	0	0	?
			Alopecurus pratensis	1	0	0	
			Calamagrostis lanceolata	1	1	2 to 3	

Some of the infections obtained on *Calamagrostis lanceolata* when inoculated with aecidiospores from *Rhamnus cathartica* are due evidently to the vars. *lolii* and *alopecuri*. These varieties are also capable of producing small uredopustules on *Phalaris arundinacea* and it seemed likely that they might have caused the slight infections obtained on this grass.

In the case of *Dactylis glomerata*, the uredospores produced as a result of inoculation with aecidiospores from *Rhamnus cathartica* were found to belong to var. *lolii*, which had been shown in the experiments with uredospores to cause heavy infection on this grass. The single plant infected by aecidiospores from *R. Frangula* did not yield sufficient spores for further inoculation to be carried out, so that it was impossible to identify the variety present. The inoculum from *R. Frangula* appeared to contain only var. *calamagrostidis* and, as this variety had been shown occasionally to produce pustules of types 1 and 2 on *Dactylis glomerata*, it was thought that it might have caused the infection in this instance.

It seems, therefore, that although the grasses in Group II are infected by aecidiospores from both *Rhamnus Frangula* and *R. cathartica*, the varieties causing the infections are different in the two cases. Text-fig. 1 indicates diagrammatically the varieties found on the two alternate hosts, and the way in which they are considered to infect the grasses of Groups I and II.

In the light of this interpretation, the results of the experiments with aecidiospores are no longer opposed to those of the experiments with teleutospores, since they indicate that vars. *holci*, *arrhenatheri*, *lolii* and



Text-fig. 1. Diagram showing the constitution of the aecidiospore collections made on *Rhamnus cathartica* and *R. Frangula* and the grasses which they infected. ——→ Variety identified by experiment. - - → Identity of variety inferred from evidence obtained in related experiments.

alopecuri have their aecidial stage on *R. cathartica*, and var. *calamagrostidis* has its aecidial stage on *R. Frangula*. They are, also, in accordance with the results of Klebahn, Eriksson and Mühlethaler, in so far as they show that the varieties of *Puccinia coronata* are markedly specialized in relation to their aecidial as well as to their uredinial hosts.

It is possible, moreover, that some of the results obtained by Treboux (1912, 1914), which appeared to be so much at variance with those of other European workers, may be explained in the same way as the infection of *Calamagrostis lanceolata* with aecidiospores from both species of *Rhamnus* has been explained in the present experiments. For example, Treboux (1912) found that *Phalaris arundinacea* was infected to some extent by aecidiospores from *Rhamnus cathartica*, although Klebahn

(1895, 1896, 1898) and Eriksson (1897, 1909) had found that rust from this grass had its aecidial stage only on *R. Frangula*. Amongst the other hosts which Treboux infected with the spores from *R. cathartica*, were *Avena sativa, Alopecurus pratensis* and *Lolium perenne*, and it is thus possible that his infections on the *Phalaris* were due to the varieties *avenae, alopecuri* or *lolii*, all of which have been shown to infect this grass.

The grasses occurring in Group III were *Agropyron repens, Bromus sterilis* and *Agrostis palustris*. They bore no pustules when inoculated with aecidiospores from either species of *Rhamnus*, although their inoculated leaves were usually flecked.

During the years in which the experiments were carried out, no crown rust was found in the neighbourhood of Cambridge on either *Agropyron* spp. or *Bromus* spp., so that the varieties corresponding to the f.sp. *agropyri* recorded by Eriksson (1897, 1909) in Hungary and Moravia and the f.sp. *bromi* recorded by Mühlethaler (1911) in Switzerland appear to be absent from Cambridgeshire. Slight infection of *Agrostis palustris* was observed in the field, but it proved impossible to cultivate the rust from this grass in the greenhouse. The variety responsible for the infection is, therefore, unknown.

(ii) *Inoculation of oat varieties.* The results of the inoculation of a number of oat varieties with aecidiospores from both species of *Rhamnus* are shown in Table IV. The plants were only slightly infected, whether

Table IV

Results obtained on inoculations of oat varieties with aecidiospores collected in the field on Rhamnus Frangula *and* R. cathartica. *Summers* 1933 *and* 1934

Variety inoculated	Aecidiospores on *R. Frangula*: Total no. of plants: Inoculated	Infected	Infection type	Aecidiospores on *R. cathartica*: Total no. of plants: Inoculated	Infected	Infection type
Avena sativa:						
Abundance	18	0	0	36	0	0
Black Tartary	18	0	0	26	0	0
Fyris	15	0	0	23	3	0, 1, 2 −
Grey Winter	16	0	0	21	1	0, 1
Hede	12	0	0	20	1	0, 1
Mesdag	12	0	0	19	1	0, 1
Scotch Potato Oat	5	0	0	9	0	0
Thousand Dollar	10	0	0	17	0	0
White Cross	12	3	0, 1	24	0	0
Avena strigosa:						
Ce 2596	18	0	0	27	2	0, 1, 2

they were inoculated with aecidiospores from *R. Frangula* or *R. cathartica* and this was somewhat surprising, as oats are frequently heavily infected by *Puccinia coronata* in this country. It appeared likely that the poor infection was due to the absence of the appropriate variety of rust in the aecidial inoculum. This was confirmed in the two cases in which sufficient uredospores were obtained for the variety causing the infection to be identified. Spores produced on Fyris by inoculation with aecidiospores from *Rhamnus cathartica* were found to belong to the variety *alopecuri*, and those produced on White Cross inoculated with aecidiospores from *R. Frangula* to belong to the variety *calamagrostidis*. Both these varieties had been shown in the experiments with uredospores to be capable of causing slight infection on oats. It appeared, therefore, that the variety *avenae* was absent from the aecidial inoculum obtained in the neighbourhood of Cambridge in 1933 and 1934 and this was confirmed by the fact that the oat crops were quite free from crown rust in those years.

Experiments on the effect of passage through the aecidial host upon the pathogenicity of varieties of *Puccinia coronata*

Dietz (1926*b*) found that the pathogenicity of certain varieties of *Puccinia coronata* was markedly altered by their passage through the aecidial host. Uredospores of var. *avenae*, for example, infected *Holcus lanatus* only slightly and *Calamagrostis canadensis* not at all, but aecidiospores of the same variety infected both grasses very heavily. Dietz could give no explanation of this apparent effect of the alternate host upon the pathogenicity of the variety but the genetical work resulting from Craigie's discovery of heterothallism in the rusts has thrown light upon this matter. Newton *et al.* (1930 *a*, *b*; 1932) have shown that the physiologic races of *Puccinia graminis tritici* are usually heterozygous for pathogenicity, and that when a race is passed through the alternate host, the segregation and recombination of the factors governing this character generally lead to the production of a number of different races, none of which may be identical with the parent. Moreover, it has been shown by Waterhouse (1929) and Newton *et al.* (1930 *a*, *b*, 1932) that when two physiologic races are hybridized on the barberry, races differing from either of the parents may be found in the aecidial progeny. Similar results have been obtained by Johnson *et al.* (1932, 1933) and Stakman *et al.* (1930, 1934) in crosses between varieties of *P. graminis*, although there is a greater degree of inter-sterility between the varieties than between the physiologic races.

It seems possible that the changes in pathogenicity obtained by Dietz in *P. coronata* were due either to the heterozygous nature of the variety used, or to accidental hybridization. In the present investigation, some experiments were carried out to determine the effect of self-fertilization and hybridization on the varieties of *P. coronata* occurring in this country.

(*a*) *Experiments on self-fertilization.* Plants of *Rhamnus Frangula* and *R. cathartica* were inoculated with sporidia from germinating teleutospores as previously described, and were placed out of doors in insect-proof cages. When the spermogonia were well developed, the "selfing" of the variety was carried out by transferring nectar from one infection pustule to another on a sterilized needle. Nectar from as many pustules as possible was intermixed so that the + and − strains had ample opportunity of coming into contact. Where the infection pustules were numerous and confluent, this mixing of the nectar was unnecessary.

In order to investigate the pathogenicity of the aecidial progeny, the aecidiospores were inoculated on to as many as possible of the series of grasses used for the differentiation of varieties in the experiments with uredospores (Brown, 1937). The inoculations were carried out with a mixture of spores from as many aecidia as were available, instead of with spores from a single aecidial cup, as in the experiments of Newton & Johnson (1932). This was done because no grass or cereal was available which was susceptible to all the varieties of the rust, and it was necessary to inoculate as many grasses as possible so as to afford any varieties or races which might arise an opportunity of infecting a congenial host.

The varieties used were *calamagrostidis*, *holci* and *alopecuri*. Aecidia of var. *calamagrostidis* were produced on *Rhamnus Frangula* by inoculation with teleutospores formed in the greenhouse on *Calamagrostis lanceolata* and *Phalaris arundinacea*; and aecidia of vars. *holci* and *alopecuri* were produced on *Rhamnus cathartica* by means of teleutospores formed in the greenhouse on *Holcus lanatus* and *Alopecurus pratensis*, respectively. Table V shows the infection types given by the aecidiospores of these three varieties compared with those given on the same grasses by their uredospores as described previously (Brown, 1937).

Close agreement is shown between the infection types given by the aecidiospores and the uredospores and, where small differences do occur, e.g. in var. *alopecuri* on *Phalaris arundinacea* and *Arrhenatherum avenaceum*, they are too small to be significant, especially when the genetic impurity of the host plants is taken into consideration. It appears, therefore, that the pathogenicity of these varieties is not appreciably altered by their passage through the aecidial host.

Table V

Infection types given by aecidiospores of vars. calamagrostidis, holci *and* alopecuri *compared with those given by their uredospores*

Grass species inoculated	var. *calamagrostidis* Aecidio-spores	var. *calamagrostidis* Uredo-spores	var. *holci* Aecidio-spores	var. *holci* Uredo-spores	var. *alopecuri* Aecidio-spores	var. *alopecuri* Uredo-spores
Calamagrostis lanceolata	4	3, 4	0	0	0, 2, 3	0, 1, 2, 3
Phalaris arundinacea	3, 4	3, 4	—	0	0, 1, 3	0, 1, 2
Dactylis glomerata	0	0, 1	—	0	0	0, 1
Alopecurus pratensis	0	0	0	0	4	4
Lolium perenne	0	0	0	0	0	0
Holcus lanatus	0	0	4	4	0	0
Arrhenatherum avenaceum	0	0	0	0	0, 1	0
Bromus sterilis	0	0	—	0	0	0
Avena sativa (var. Abundance)	—	0	—	0	0	0, 1
No. of experiments carried out with aecidiospores of each variety	4		2		4	

Table VI

Infection types produced by aecidiospores on Rhamnus Frangula *and* R. cathartica *infected by teleutospores collected in the field*

Grass species inoculated	Source of teleutospores: *Calamagrostis lanceolata*	*Phalaris arundinacea*	*Holcus lanatus*	*Arrhenatherum avenaceum*
Calamagrostis lanceolata	3, 4	3, 4	0	—
Phalaris arundinacea	3	3, 4	—	0
Dactylis glomerata	0, 2	0, 2	—	-
Alopecurus pratensis	0	0	0	—
Lolium perenne	0	0	0	0
Holcus lanatus	0	0	4	0
Arrhenatherum avenaceum	0	0	0	4
No. of experiments carried out with aecidiospores from each source	4	5	2	2

Experiments were carried out with the aecidiospores produced on the two *Rhamnus* species when inoculated with teleutospores collected in the field on *Calamagrostis lanceolata*, *Phalaris arundinacea*, *Holcus lanatus* and *Arrhenatherum avenaceum*, and the results are shown in Table VI. Although, in some cases the aecidial material was very scanty, the infection types produced agree with those given by the uredospore cultures of the varieties appropriate to the grasses bearing the teleutospores. Aecidiospores produced by inoculation with teleutospores on *Calamagrostis lanceolata* and *Phalaris arundinacea*, give infection types

similar to those of uredospore cultures of var. *calamagrostidis*; and aecidiospores produced by inoculation with teleutospores on *Holcus lanatus* and *Arrhenatherum avenaceum* give infection types similar to those of uredospore cultures of vars. *holci* and *arrhenatheri* respectively. These experiments indicate that the varieties of *Puccinia coronata* are not altered appreciably by their passage through the aecidial host.

(*b*) *Experiments on the hybridization of varieties*. The technique employed in these experiments was essentially the same as that used by Newton & Johnson (1932) for the hybridization of physiologic races of *Puccinia graminis tritici*. Isolated spermogonial pustules were chosen and nectar from the pustules of one variety was transferred to those of another on the point of a sterilized needle. Each pustule received nectar from several pustules of the variety with which it was being crossed and the needle was sterilized between each transfer. Whenever possible reciprocal crosses were made and in each experiment some of the pustules were "selfed", i.e. nectar was transferred to them from other pustules of the same variety. Crosses were made between the following varieties: *calamagrostidis* × *holci*, *calamagrostidis* × *alopecuri*, *holci* × *alopecuri* and *holci* × *lolii*. Out of ten crosses made, aecidia were obtained only in four and, in these four, only on seven out of the nineteen "crossed" pustules. On the "selfed" pustules aecidia were obtained in every case except one. The infection types produced on certain grasses by the aecidiospores from the "crossed" pustules agreed so closely with those produced by aecidiospores from the "selfed" that it seems likely that the former were not of hybrid origin, but arose through accidental selfing. These varieties of *Puccinia coronata* do not appear to hybridize readily and resemble in this respect the varieties of *P. graminis* investigated by Johnson *et al.* (1932) and Johnson & Newton (1933).

DISCUSSION

The results obtained show that the varieties of *Puccinia coronata* occurring in Great Britain are markedly specialized in relation to their alternate hosts. The varieties *alopecuri*, *avenae*, *arrhenatheri*, *festucae*, *holci* and *lolii* have their aecidial stage on *Rhamnus cathartica* and do not infect *R. Frangula* and the variety *calamagrostidis* has its aecidial stage on *R. Frangula* and only occasionally infects *R. cathartica*, with the production of abortive spermogonia.

Klebahn (1892) used this difference in aecidial host relationship as a basis for dividing the original species, *Puccinia coronata* Corda, into two; *P. coronata* Kleb. and *P. coronifera* Kleb., but, in the light of our present

knowledge of specialization of parasitism in the rust fungi, it is doubtful if such a difference should be used as a specific criterion. The definition of a species employed by Arthur in his *Manual of the Rusts in United States and Canada* (Purdue Research Foundation, 1934) is that formulated by the American Phytopathological Society in 1925 (*Phytopathology*, **15**, 316). It appears to be generally accepted by mycologists and is as follows: (The term species shall be applied to) "a group of individuals which can be segregated on the basis of morphologic characters of such a nature as to be applicable and determinable by mycologists and pathologists in general and such as will be available for general, taxonomic purposes." Cultural characters and differences in host relationship are not used as criteria for species differentiation, and as there are no morphological characters whereby the varieties of crown rust infecting *Rhamnus cathartica* can be distinguished from those infecting *R. Frangula*, there seems to be no reason why they should be considered to belong to different species. The problem is merely one of physiologic specialization involving the aecidial as well as the uredinial stage and, if a difference in the aecidial host were used as a specific distinction, the varieties of crown rust which occur in Canada and the United States and have their aecidia on *Rhamnus alnifolia*, *Lepargyrea canadensis* and *Eleagnus commutatus* should all be considered as separate species.

A practical difficulty in dividing crown rust into two species is the impossibility of assigning a collection of rust made in the field either to *Puccinia coronata* Kleb. or to *P. coronifera* Kleb. (*P. Lolii* Niels.) without carrying out cultural experiments. It is not sufficient to take the uredinial host on which the rust is found as an indication of the species to which it belongs, because the present experiments have shown that a "*coronifera*" variety of rust may cause quite heavy infection on a "*coronata*" host. For example, var. *lolii* may cause an infection of type 3 upon *Calamagrostis lanceolata*, so that if rust is found on this grass in the field, it is impossible to tell without experiment whether it belongs to var. *calamagrostidis*, which has its aecidial stage on *Rhamnus Frangula*, or to var. *lolii*, which has its aecidial stage on *R. cathartica*. It would seem to be in the interests both of accuracy and of convenience if the use of the specific names *Puccinia coronata* Kleb. and *P. coronifera* Kleb. (*P. Lolii* Niels.) were discontinued and the original name *P. coronata* Corda were employed to designate all those types of rust which have in common the coronate teleutospores and other morphological characters which we associate with crown rust.

Summary

1. Experiments were carried out to determine the relationship between the varieties of *Puccinia coronata* Corda previously isolated in Great Britain and the two species of *Rhamnus*, *R. Frangula* and *R. cathartica*, which are the alternate hosts of the rust in this country.

2. The experiments included: (*a*) the inoculation of seedling plants of the *Rhamnus* species with sporidia from germinating teleutospores of the different varieties; and (*b*) the inoculation of a number of grasses with aecidiospores collected in the field on the two species of *Rhamnus*.

3. The varieties were found to show a considerable degree of specialization in their relation to the alternate hosts, vars. *alopecuri*, *arrhenatheri*, *avenae*, *festucae*, *holci* and *lolii* producing aecidia on *R. cathartica* only and var. *calamagrostidis* on *R. Frangula* only.

4. The difference in aecidial host relationship of the varieties is not considered to be an adequate criterion for species differentiation, and it is suggested that the use of the names *Puccinia coronata* Kleb. and *P. coronifera* Kleb. (*P. Lolii* Niels.) be discontinued and the rust be designated by the original name *P. coronata* Corda.

5. The pathogenicity of the varieties was not altered appreciably by passage through the alternate host and the varieties did not appear to hybridize readily.

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Fig. 1

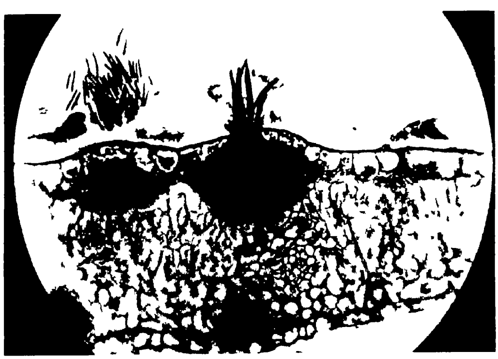

Fig. 2

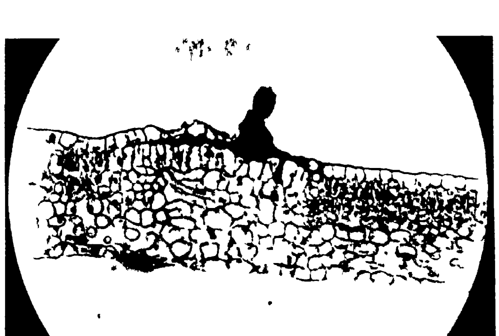

Fig. 3.

BROWN.—A Study of Crown Rust, *Puccinia coronata* Corda, in Great Britain (pp. 506–527)

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EXPLANATION OF PLATE XXI

Fig. 1. Leaves of *Rhamnus Frangula* and *R. cathartica* inoculated 30. v. 34 with teleutospores on *Phalaris arundinacea*. Photograph taken 21. vi. 34. Natural size. Leaf (*a*) *Rhamnus Frangula*, upper surface showing numerous infection pustules. Leaf (*b*) *R. Frangula*, lower surface showing aecidia. Leaf (*c*) *R. cathartica*, upper surface showing shrivelled infection pustules with abortive spermogonia. Leaf (*d*) *R. cathartica*, lower surface showing shrivelled infection pustules with abortive spermogonia.

Fig. 2. Transverse section of leaf of *Rhamnus Frangula* inoculated with teleutospores on *Phalaris arundinacea*, showing normal spermogonium and absence of necrosis in host cells. × 170.

Fig. 3. Transverse section of leaf of *Rhamnus cathartica* inoculated with teleutospores on *Phalaris arundinacea*, showing abortive spermogonium and necrosis of host cells. × 170.

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SOME SPECIES OF *PYTHIUM* PARASITIC ON WHEAT IN CANADA AND ENGLAND[1]

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(With Plate XXII and 2 Text-figures)

The large number of publications dealing with *Pythium* parasites on the roots of graminaceous hosts in widely separated parts of the world during the last decade may be taken as evidence of the economic importance of this group of organisms. The crops most particularly affected are wheat in Canada, Italy, India, and Japan; maize in the United States, the Philippines, Hawaii, and Italy; sugar-cane in the U.S.A., Hawaii, the Philippines, Mauritius, and India; rice in Java, Japan, Portugal, and the U.S.A.; grasses and turf in the U.S.A. and Holland, as well as in many other countries. *Pythium* root rots or seedling blights have been reported from time to time on oats from Britain, Canada, Denmark, the U.S.A., and Holland; on barley from Tunis, Canada, and Japan; and on milo (*Sorghum* sp.) from the U.S.A. Earlier literature reviews on the subject, together with the investigations of the root rot on cereals, are to be found in previous papers by Vanterpool *et al.* (1930, 1932, 1935).

The author studied certain *Pythium* parasites of wheat in England during the greater part of the winter months of 1935–6. The species obtained were compared with species ordinarily considered as the primary parasitic agents in browning root rot of wheat on the Canadian prairies. The work was completed after the author's return to Canada. The combined results are embodied in this paper.

Pythium damage has attracted little attention until comparatively recently. In so far as the root rot of cereals is concerned, diseased plants resemble, and have doubtless been mistaken for, plants suffering from drought, from excess salts or alkali, or from nutrient deficiencies, and in the case of winter cereals, from prolonged wet, cold, dull weather. Indeed, there is evidence that with continuous cropping or improper crop

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rotation and farm practices, nutrient deficiencies or unbalanced nutrition do predispose the host plants to attack by the parasite (Carpenter, 1934; Cooke, 1934; Vanterpool, 1935). Difficulty, too, is commonly experienced in isolating *Pythium* parasites of roots when these are known definitely to be present in diseased portions of the root. The recognized method (Rands & Dopp, 1934; Vanterpool & Truscott, 1932) for isolating phycomycetous root pathogens is likely to give best results and should be employed. A difficulty frequently confronts the investigator in this field. The rather limited cultural requirements of many species for the formation of sexual organs and for the initiation of zoospore discharge make it often difficult to obtain oogonia and antheridia, and zoospores, in culture. The specific identity of the fungus is, therefore, either not ascertained or greatly delayed.

It is perhaps significant that, with few exceptions, all the species concerned belong to the nematosporangial group of *Pythium*, the most important being *P. arrhenomanes* Drechsl., considered in its broader sense, and *P. graminicolum* Subram.

METHODS

In the investigation in England, wheat-seedling material from two sources was used for isolation purposes. In one case wheat was grown in pots of soil collected the previous summer from fields in wheat-growing districts near Slough, Cambridge, and Ramsgate. These soil samples, which were kindly given to me by Mr S. D. Garrett, had been air-dried for a considerable time. After five or six weeks the seedlings were washed free of soil and many root lesions were found to contain *Pythium* oospores. The subsequent isolation procedure was according to the method previously stated. In the second instance, wheat seedlings with root systems and adhering soil were procured from fields at Harpenden, Reading, Jealott's Hill, and Littlewood, during the last week of March 1936, and cultures obtained as before. Then followed a preliminary cultural study in which only one or two strains of each morphologically similar form from one source were kept. The pathogenicity of these isolates to wheat was then conducted in small Erlenmeyer flasks as described in a previous paper (Vanterpool & Truscott, 1932). Those strains showing parasitic ability were reserved for further cultural studies and pathogenicity tests on wheat in sterilized potted soil. Maize-meal agar, carrot maize-meal agar, and water agar containing wheat root tips were the media used in the comparative morphological study in Petri dishes, the last-named medium being particularly suitable for the development and study of

oogonia and antheridia, especially in the vicinity of the wheat root tips. All growth rates were obtained from colonies on carrot maize-meal agar. Water cultures, with and without steam-sterilized wheat root tips, were used mainly for zoospore discharge, but were also supplementary to the solid media.

Pathogenic species

(1) *Pythium arrhenomanes* Drechsl. (Drechsler, 1928, 1936; Rands & Dopp, 1934)

This species is here considered in the wider sense of Rands & Dopp (1934) to include those forms morphologically similar in the radiating aspect of the numerous diclinous antheridial stalks, regardless of whether or not lobulate sporangia have been observed. It is very widely distributed and probably causes more damage to graminaceous crops than any of the other species under consideration. It has been reported as the cause of more or less severe root rots of maize in the U.S.A. (Johann *et al.* 1928), the Philippines (Roldan, 1930, 1932), Hawaii (cf. Sideris, 1931) and Canada (unpublished information); of wheat in Canada (Vanterpool & Truscott, 1932; Vanterpool, 1935), and Hawaii (cf. Sideris, 1931); of sugar-cane in the U.S.A. (Edgerton *et al.* 1929; Rands & Dopp, 1934), Hawaii (cf. Drechsler, 1936; Rands & Dopp, 1934), the Philippines (Roldan, 1930), India (Subramaniam, 1936), and Mauritius (Shepherd, 1933), and of milo (*Sorghum* sp.) in the U.S.A. (Elliott *et al.* 1932, 1937).

Two English strains were obtained: one from wheat seedlings grown in soil, which had been air-dried for several weeks, from plots at the Rothamsted Experimental Station, and the other from seedlings collected from a clay-loam soil in a field near Littlewood, in April 1936. The Rothamsted soil was procured through the kindness of Dr J. B. Marshall, and the Lincolnshire sample through that of Mr R. V. Tipler. The Rothamsted strain was readily obtained from soil which had received ammonium sulphate fertilizer only, or ammonium sulphate plus minerals, over a number of years. Both the English strains were larger in average spore size than the Canadian type culture (Vanterpool *et al.* 1932) and failed to produce (lobulate) sporangia; they closely resembled other non-lobulate, large-spored Canadian strains which were available for study. The Rothamsted strain had a growth rate approximately equal to that of the Canadian type culture, while the Lincolnshire strain had a lower growth rate. Both the English strains proved to be highly pathogenic to Marquis wheat.

(2) *Pythium graminicolum* Subram. (Subramaniam, 1928; Drechsler, 1936)

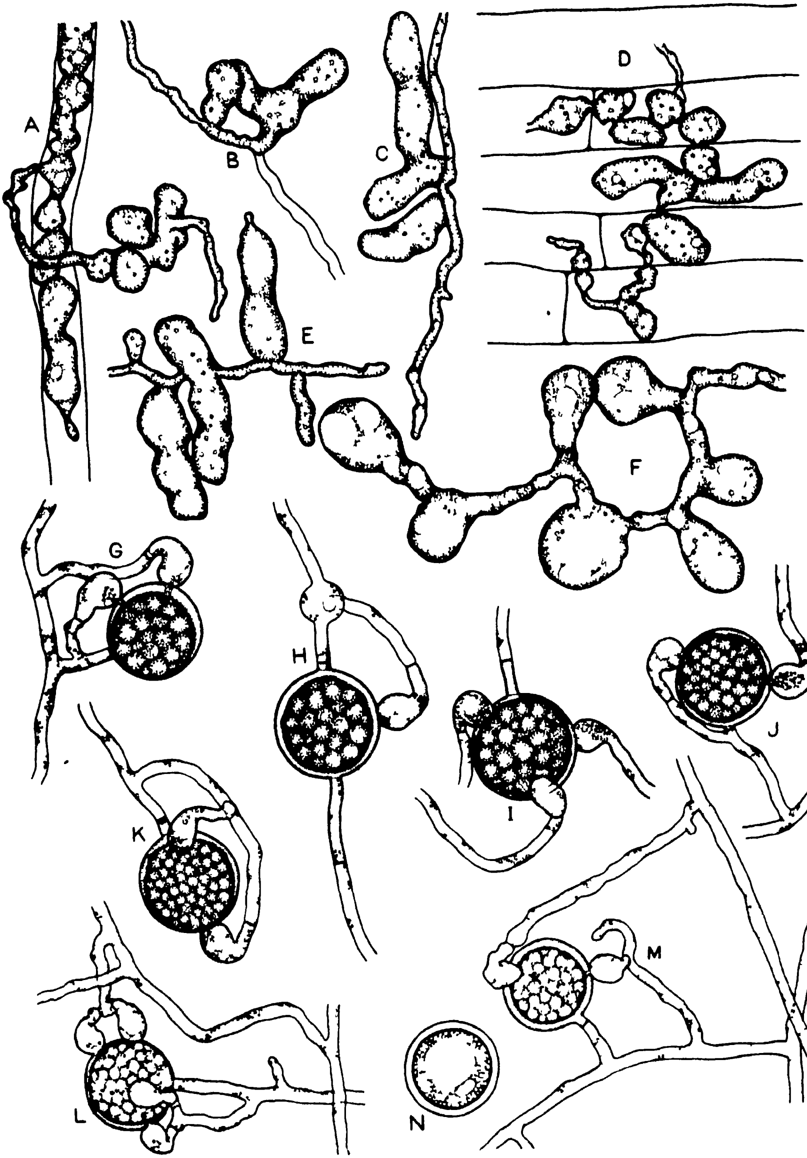
This species was first described as a parasite of wheat in India (Subramaniam, 1928) and is frequently obtained from rotting sugar-cane roots in the U.S.A. (Rands & Dopp, 1934), Porto Rico (Drechsler, 1936), India (Subramaniam, 1936), and Hawaii (Carpenter, 1934; cf. Drechsler, 1936) where, together probably with *P. arrhenomanes*, it is one of the chief factors in the decline or failure of certain sugar-cane varieties. Confusion arising between the identity of this species, the original type culture of which is no longer available, and *P. arrhenomanes* has recently been cleared up by Drechsler (1936). The writer has not encountered any forms in Canada which he considers to belong to this species.

The English strain was isolated in December 1935 from wheat seedlings grown in field soil collected the previous summer near Cambridge. In the interval the soil had become thoroughly air dried. In spore size this strain is more in agreement with Subramaniam's plant (1928) than with Miss Matthew's (1931). It was more pathogenic to Marquis wheat than a strain from sugar-cane received from Dr C. Drechsler six years ago.

(3) *Pythium volutum* Vanterpool & Truscott (1932; Van Luijk, 1934)

This species was first reported as a parasite of wheat and oats in Canada (Vanterpool & Truscott, 1932) and later of grasses in Holland (Van Luijk, 1934). Though closely resembling its congener *P. arrhenomanes*, the writer does not consider that it falls within the ambit of the latter species as defined by Rands & Dopp (1934). Its chief characteristics are sufficiently distinct to warrant specific rank. It differs from *P. graminicolum* chiefly in having its oospores usually free within the oogonium, in producing sporangia rarely, and in being less frequently androgynous.

Two English strains, agreeing closely with the Canadian type species in general cultural characters, in antheridial disposition, and in the wrapping of antheridial branches about the oogonial stalk under certain cultural conditions, were obtained in December 1935 from wheat seedlings in potted soil; one from soil collected near Ramsgate, and the other from Slough soil. These were farm soils typical of the districts, with reactions of pH 8·0 and 6·8, respectively. The English strains are severely parasitic on wheat seedlings.



Text-fig 1

(4) *Pythium tardicrescens* n.sp. (Pl. XXII, figs. 1, 2 and 4; Text-fig. 1)

This form is found both in Canada and England. It was first isolated in Canada in 1929, from wheat roots affected with browning root rot and is now considered next in importance to *P. arrhenomanes* among the causal agents of this disease. Over a number of years it has been obtained from widely separated points in the wheat-growing area of Saskatchewan. It is slow-growing and exacting in its cultural requirements and is not as readily isolated as some of the other species. No growth occurs at 30° C. It is further characterized by the clumping of the oogonial contents into large, lustrous oil drops which stain red with Sudan III. Only a small percentage of oospores mature. Though not as actively pathogenic to wheat under artificial conditions as *P. arrhenomanes* and *P. aristosporum*, it produces brown root lesions strikingly similar to those obtained under field conditions (Pl. XXII, fig. 2). Oospores are found scattered sparsely through these lesions, occasionally even in the tracheae.

P. tardicrescens has many morphological and cultural characteristics in common with *P. sclerotcichum* (Drechsler, 1934), but is distinctly different from this form in its much slower growth rate and in the production of lobulate diverticula.

The English strain was isolated in November 1935 from Slough soil, *p*H 6·8, by means of wheat roots. The two strains approximate each other closely.

Description

Mycelium with a flat, radiate, non-lustrous growth on agar; hyphae mostly 2·5-5 μ. in diameter, and irregularly branched with fine laterals, knob-like appressoria being often present; radial growth on carrot maize-meal agar about 11 mm. in 24 hr. at 22° C.

Toruloid buds or a moderate development of lobulations infrequently produced in old cultures on the surface of plain agar containing wheat root tips, or in sterile water root-tip cultures, and observed intracellularly in living tissues; buds never complex, rarely exceeding 12 μ. in diameter; zoospore discharge not observed, instead, germ

Text-fig. 1. *Pythium tardicrescens*. Showing lobulate elements and sexual apparatus; drawn with the aid of a camera lucida. × 700. A. More or less toruloid lobulations within a root hair, with an external portion showing slightly more extensive development. B, C, E and F. Lobulate diverticula developed on old water-agar plates containing wheat root tips. D. The same as seen in the cortical cells of a wheat root in water culture. G, H, K. Oogonia with monoclinous antheridia. Note the characteristic lumpy nature of the oogonial contents. H, an intercalary oogonium. I. Diclinous apparatus. J, L, M. Oogonia with both monoclinous and diclinous antheridia. N. A mature oospore.

tubes are produced which may terminate in a dark conidium; spherical walls of germinated conidia frequently conspicuous in culture.

Oogonia smooth, terminal on short branches or rarely intercalary, commonly with large, lustrous oil globules, 17–30 μ. (average 24·1 μ.) in diameter, forming readily in plain agar containing wheat root tips or in water root-tip cultures, but more sparsely on the agar medium alone; empty oogonial "shells" common following failure to mature though occasionally oogonia become filled with tangled hyphae; delimiting septum frequently visible outside the edge of the oogonium. Antheridia up to six, but usually two to three; club-shaped or crook-necked, averaging 6–8·5 μ. in width, 10·5 μ. from apex to curve and 5·5 μ. from curve to septum, with a fertilization tube of 2 μ. and making fairly narrow to medium contact with the oogonium; they commonly arise from the oogonial stalk or a branch, but all may come from neighbouring branches, each of which may supply two or, less often, three.

Oospores, smooth, spherical or subspherical, usually free within the oogonium, 16–26 μ. (average 20·3 μ.) in diameter, with a central globule averaging 11·1 μ. usually clear and embedded in a finely granular matrix and a wall 1·25–2 μ.; they form best in solid or liquid cultures containing wheat root tips.

Causes a root rot of wheat in Saskatchewan, Canada, and in England; also pathogenic to other cereals and certain grasses when artificially inoculated; original isolation and type culture from diseased roots of *Triticum aestivum* L., Saskatchewan, 1929.

Pythium tardicrescens n.sp.

Mycelium, in agaro cultum, prostrate, sine fulgore et tarde crescens. Hyphae praecipuae plerumque 2·5–5 μ. latae, ramos angustiores et frequenter appressoria bullata gerentes; in culturis vetustis, etiam in radicibus Tritici vivis, toruloide ampliores, vel in diverticula subsimpliciter lobulata, ad 12 μ. lata, productae. Conidia globosa, opaca, in apice tubi singulatim orientia. Oogonia globosa, levia, in ramis brevibus acrogena vel rare intercalaria, guttulis oleosis magnis nitentibus repleta (nisi vero propter frustrationem vacua), 17–30 μ. in medio 24·1 μ. diam. Antheridia saepius 2–3, etiam 6, frequentius e stipite vel ramo oogoniali, rarius ex hyphis alienis, orientia, clavata vel cervicem curvata, prope 6–8·5 μ. lata, 16 μ. longa i.e. 10·5 μ. ex apice ad partem curvatam et 5·5 μ. ab hac ad septum; graciliter vel modeste cum oogonio conjuncta; tubo ubertatis 2 μ. lato praedita. Oosporae leves, globosae vel subglobosae, plerumque singulae intra oogonium ejus liberae, 16–26 μ., in medio 20·3 μ. diam., globulo centrali, saepius pellucido, in matrice granulari incluso, et exospora 1·25–2 μ. crassa praeditae.

Hab. Parasitica in radicibus Tritici aestivi L., prope Saskatchewan, Canada. 1929 (Typus); postea frequenter in Canada et in Anglia inventa.

Obs. A speciebus affinibus vel commixtis differt mycelio tardicrescenti diverticulis lobulatis praedito, et guttulis oogoniorum oleosis.

(5) *Pythium aristosporum* n.sp. (Pl. XXII, figs. 3, 5–7; Text-fig. 2)

This plant has been found in Canada only, being first isolated from diseased wheat roots in 1930, in Saskatchewan, and rarely encountered since. It is characterized by the ready production of abundant mature oospores in culture, the percentage of aborted oogonial remains being

small when compared with other congeners. Occasional peculiarities are the presence of a large bulge in the oospore wall (Pl. XXII, fig. 6, and Text-fig. 2 L), and two malformed oospores within one oogonial case, one spore being usually quite small and sometimes appearing as a disorganized lump of crushed protoplasm (Pl. XXII, fig. 7 and Text-fig. 2 K). It is closely allied to *P. graminicolum* and *P. myriotylum*, but in parallel cultures can readily be distinguished by the characteristics mentioned. Like *P. graminicolum* the collapsed oogonial wall with adhering antheridia persist for a considerable time after the oospores have matured (Text-fig. 2 H). Lobulate elements develop later in culture and are not as extensive as in those two species; germination by zoospores has not been induced. The antheridial elements more often arise in closer proximity to the oogonium than is the case in *P. myriotylum*, in comparison with which it also has larger oogonia and oospores. It is aggressively parasitic on the roots of wheat, and moderately so on oats, barley and rye.

Description

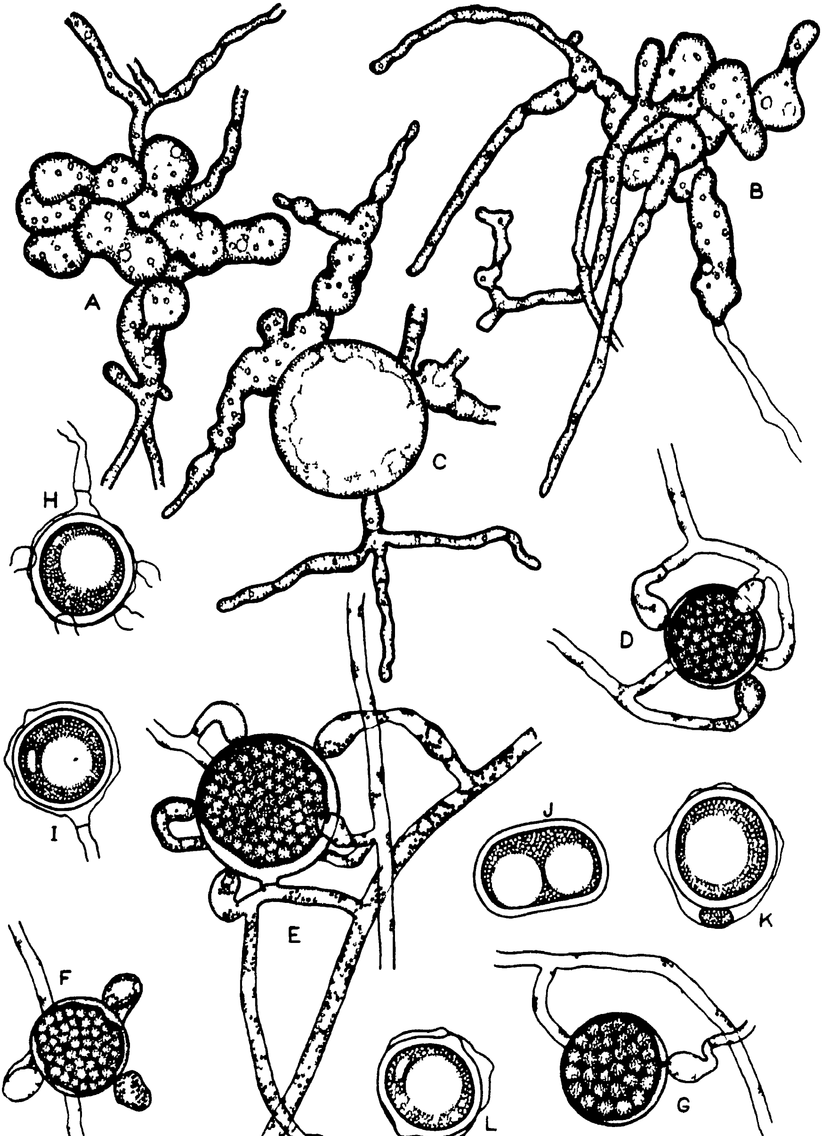
Mycelium somewhat lustrous with slight aerial development, soon becoming granular in appearance and creamy in colour, with hyphae 2·5–6·5 μ. in diameter and numerous appressoria; radial growth on carrot maize-meal agar 22–25 mm. in 24 hr. at 22° C.; conidia up to 40 μ. in diameter sometimes present, germinating by one or more germ tubes, irregular in course and soon branching.

Lobulations develop in due course in old water cultures; at first the individual elements are digitate, but in older complexes they are more often swollen lumps; germination is by numerous tubes, zoospore production not being obtained.

Oogonia smooth, subspherical, terminal, or intercalary, most often on short side branches, abundant, 21–36 μ. (average 28·8 μ.) in diameter; they form in 2–3 days on almost any media, both extra and intramatrically; septum usually some distance away from the oogonial wall, which persists long after maturation of the oospore. Antheridia usually three to six but as many as eight or more, club-shaped or crook-necked, moderately narrow, with narrow to medium contact, 6·8 μ. wide by 10·4 μ. from point of contact to curve and 6–7 μ. from curve to septum, frequently forming entanglements about the oogonium; a single branch may supply as many as four antheridia; vegetative prolongations from antheridia rarely observed; androgynous and diclinous.

Oospores smooth, subspherical, deep brown, usually free within the oogonium, abundant on most substrata and 13–30 μ. (average 24·2 μ.) in diameter, but with considerable range in size, with a central globule mostly 12–14 μ., a refringent spot 6 by 2·3 μ. and a wall about 1·5–2 μ.; germination by one or more germ tubes. Oblong oospores with as many as four reserve globules are occasionally observed in wheat roots, but more often oospores with one or two rounded lumps on their walls are present. Two oospores, either or both of which may be malformed, are sometimes encountered within a single oogonial case.

Isolated from diseased roots of wheat in Saskatchewan, Canada, 1930; also parasitizes other cereals and various grasses.



Text-fig 2

Pythium aristosporum n.sp.

Mycelium nonnihil nitens, minime aereum, mox granulare et sufflavum. Hyphae 2·5–6·5 μ. latae, appressoria numerosa gerentes; tempestive, in culturis aqueis, primo digitate- dein amorphe-lobulatae, zoosporis carentes, et tubis pluribus praeditae. Conidia 40 μ. diam., tubis germinalibus, singulis vel pluribus, vagis, mox ramosis instructa. Oogonia levia, subglobosa, plerumque a septo satis distanti limitata, acrogena vel intercalaria, frequentius in ramo brevi orientia; in omnibus mediis culturalibus per diem secundam vel tertiam numerosissime producta; membrana exteriori, longe post oosporae maturitatem manifesta, instructa; 21–36 μ., in medio 28·8 μ., diam. Antheridia 3–6, etiam 8, (ad 4 in ramo singulo orientia) androgyna et diclina, clavata vel cervicem curvata, modice tenuia, cum oogonio graciliter vel modeste conjuncta, 6·8 μ. lata, 17 μ. longa, i.e. 10·4 μ. ex apice ad partem curvatam, et 6–7 μ. ab hac ad septum; circum oogonium frequenter implicata. Oosporae leves, subglobosae, brunneae, singulae saepius intra oogonium ejus liberae, in substratis pluribus numerosae, plerumque 13–30 μ. in medio 24·2 μ., diam.; globulo centrali saepius 12–14 μ. diam., loculo refringenti 6 × 2·3 μ., et exospora 1·5–2 μ. crassa typice instructae. Oosporae in statibus abnormalibus interdum visae, (i) oblongae et 1–4 guttulatae (in radicibus Tritici vivis observatae); (ii) geminae, frequenter deformatae, in oogonio singulo locatae; (iii) tumoribus exosporae singulis vel duobus ornatae.

Hab. Parasitica in radicibus Tritici aestivi, prope Saskatchewan, Canada.

Obs. A speciebus affinibus P. graminicolo et P. myriotylo differt praecipue oogoniis maturis, in omnibus mediis culturalibus facile et numerose productis; minus et infrequentius, tumoribus exosporae et oosporis geminis.

(6) *Pythium torulosum* Coker & Patterson [?], (Coker & Patterson, 1927; Matthews, 1931; Van Luijk, 1934)

A species of *Pythium* in general agreement with the description of *P. torulosum* Coker & Patterson was isolated at least twice by Miss Mary D. Glynne, Rothamsted Experimental Station, from lesions on the stem bases of wheat plants in March 1936. The writer, about the same time, obtained it in pure culture from the roots of wheat seedlings from the same source, and somewhat earlier from the roots of seedlings from Reading. The English form is, also, probably in closer agreement with a fungus of the "*gracile*" group described by Petri (1930) as attacking mainly the basal portions of the stems of wheat plants in the province

Text-fig. 2. *Pythium aristosporum* developed in water culture containing wheat root tips; drawn with the aid of a camera lucida. × 700. A and B. Lobulate structures developed infrequently in old cultures germinating by germ tubes. C. A conidium germinating and producing ordinary germ tubes and swollen diverticula. D, E, F and G. Monoclinous and diclinous sexual apparatus. H. A mature oospore with persistent oogonial and antheridial remains. I. The same without antheridial remains. J. An oblong oospore with two central globules, developed in a root cell. K. A mature oospore with a flattened lump of protoplasm between it and the old oogonial wall. L. A mature oospore with two protuberances on the oospore wall; oogonial remains are still present.

of Padua, Italy, in 1930. In 1934, in the Netherlands, van Luijk (1934) obtained from grass roots a fungus which he regarded as *P. torulosum.*

English strain. Mycelial growth vigorous with tendency to develop aerially. Approximate radial growth rate on carrot-maize meal agar in 24 hr.: 8 mm. at 15° C., 13 at 20°, 15·5 at 25°, 4 at 30°, 0 at 35°. Sporangia made up of swollen or bulbous portions of the mycelium in a catenulate manner, seldom extensively digitate or lobulate. Zoospore discharge readily obtained. Zoospores 7·5 μ. average, when rounded. Oogonia (average 18·1 μ.), smooth, spherical, terminal or intercalary, form quickly in culture (second day). Antheridia one to two, more often only one, club-shaped, androgynous, arising usually from the oogonal stalk and disappearing after fertilization has been effected. Oospores abundant on most media, spherical, 16·8 μ. average, central globule 6·5–7·5 μ. average, with thick oospore wall, 2 μ. or more, completely filling the oogonium.

Van Luijk's culture from the Centraalbureau was studied comparatively with the English strain. Van Luijk's form has the tendency to produce a flat, rosette type of growth on rich media on agar plates, whereas the English form has a strictly radiate habit with a tendency to aerial development. Practically all types of relationships of antheridia to oogonia which have been found in one have also been observed in the other, though the types of relationships found most commonly in the respective forms differ markedly. It is characteristic of the English form for a single club-shaped antheridium to arise approximately 25 μ. down the oogonial stalk while, in van Luijk's form, it is usual for the antheridium to arise in closer proximity to the oogonium. The oospore in the English form averages 2·5 μ. larger, while the hyaline wall is twice (2 μ.) as thick as in van Luijk's form (1 μ.). Sporangia and zoospores are similar. The English form is slightly pathogenic to wheat, but van Luijk's form gave no evidence of parasitism. Van Luijk obtained a stimulation in the growth of grasses inoculated with his form (1934).

In comparison with the description and illustrations of *P. torulosum*, the English form rarely produces as complex lobulations and its oospore wall is much thicker (2 μ.) than that figured by Coker & Patterson. These authors remark on the thick nature of the oospore wall, but give only 0·8 μ. as the measurement. The oospores of the English strain show a closer resemblance to those figured by Matthews (1931, Pl. 6, fig. 5). In thickness of oospore wall van Luijk's form agrees well with Coker & Patterson's plant; the same is true for the oospore size. The oospores of Petri's form are somewhat larger, 16–24 μ., than any of the others. Petri did not obtain zoospore formation from the toruloid elements,

though this phenomenon is readily procured in both van Luijk's and our form.

The facts available seem to suggest that the English form is probably the same as the Italian. Both showed a tendency to attack the basal portion of the stems of wheat seedlings; van Luijk's form on the contrary was not pathogenic. The rosette habit of growth of van Luijk's form, together with the differences enumerated above, separate this form from the English one, to the extent of their being different varieties at least. It should be noted that neither Coker & Patterson (1927) nor Miss Matthews (1931) referred to a rosette type of growth in their plant. It is not an easy matter to settle the identity of our form under the circumstances, as neither the original type culture of *P. torulosum* nor that of Petri's fungus is available now. For the present it seems best to consider the English and the Italian forms as geographic strains of *P. torulosum*, even though it may broaden the concept of this species.

P. torulosum has not been recorded from Canada. When compared with the other species treated in this paper, the English strain is only slightly to moderately pathogenic to wheat seedlings.

GROWTH RATES

Table I shows the comparative growth rates, in radial increase in millimetres during the second 24 hr. on carrot maize-meal agar plates, of the species of *Pythium* isolated from wheat.

Table I

Radial increment in millimetres in 24 hr. on carrot maize-meal agar plates

	Canadian strain					London, May, 1936 English strain				
Organism	15° C.	20° C.	25° C.	30° C.	35° C.	15° C.	20° C.	25° C.	30° C.	35° C.
Pythium arrhenomanes	9·5	18	22	24 +	Trace	10	17·5	22	25 +	Trace
P. graminicolum	–	–	–	–	–	6·5	10	11	0	0
P. volutum	9	12	13	Trace	0	9	12	13·5	Trace	0
P. tardicrescens	3·3	8	10	Trace	0	2	6·5	7·5	0	0
P. aristosporum	10	18	23	24 +	Trace	—	—	—	—	—
P. torulosum [?] (English strain)	—	—	—	—	—	9	13	15	3	0

DISCUSSION

A striking fact in this investigation is the relative ease with which the better known *Pythium* species pathogenic to wheat could be obtained either from wheat seedlings in pots of soil collected at random from virtually any field with cereals in the rotation, or from seedlings collected

from the poorer looking parts of fields in March. It gives a probable indication of the wide distribution of these forms in the wheat fields of England. They are doubtless indigenous on native grasses and capable of attacking introduced varieties. Experiments conducted at the University of Saskatchewan have shown that species of fifteen genera of grasses are attacked when grown in naturally infested soil. The damage they cause in England is not known, but as the species of *Pythium* most pathogenic to wheat in other parts of the world have been shown to be present in five counties (Bucks, Cambs, Kent, Herts, and Lincs),[1] it is reasonable to infer that they account for a reduction in yields of wheat or other cereal which has, hitherto, been attributed to other causes. Of the six species, three are common to both Canada and England, namely, *P. arrhenomanes*, *P. volutum*, and *P. tardicrescens*; two were found in England and not in Canada, namely, *P. graminicolum* and *P. torulosum* [?], and one in Canada only, *P. aristosporum*.

Another noteworthy fact is that the six species under discussion belong to the group with lobulate sporangia. Numerous sphaerosporangial forms were isolated, but these invariably proved non-pathogenic or weakly pathogenic. These latter fail to produce brown discoloration and necrosis of the root tissues; they do, however, cause a retardation of growth in the length of the main seminal roots, with the subsequent development of an excessive number of fine laterals. It is possible that this is due to some toxic product excreted by the fungus (cf. Vanterpool, 1933). If this should be so, their presence in the soil may increase the liability of the roots to attack by the parasitic species themselves.

By the method of isolation used in these investigation in England and noted earlier in this paper, 65–90% of the isolates from discoloured root lesions are species of *Pythium* and the remainder mainly *Fusarium* spp. Of the *Pythium* isolates 30–50% are parasitic and may belong entirely to one species or to as many as three, depending on the source of the wheat seedlings. Usually, a given sample of seedlings from the field yielded two or three parasitic species, while the sample from pots gave one or two parasitic species. This demonstrates that in a comprehensive study of the fungal flora of the roots of any crop plant, use should be made of a method, such as the one employed in this investigation, of obtaining in culture the phycomycetous fungi present; otherwise, the forms obtained in culture are not representative of those occurring in the roots.

[1] More recently, May 1937, Mr S. D. Garrett, Rothamsted Experimental Station, in a written communication informed me of *Pythium* damage to wheat seedlings in Yorkshire during March, following a wet period.

It is not an easy matter to assess the parasitic vigour of the various species because of the difficulty of obtaining uniform distribution of the active inoculum throughout the soil. This is particularly true of forms such as *P. tardicrescens*, which are more limited in their cultural requirements but which, under their optimum conditions, may produce symptoms strikingly like those produced under field conditions (Pl. XXII, fig. 2). Experience has shown that the preliminary laboratory tests on parasitism conducted in small flasks, as described previously, give as trustworthy an indication of relative parasitic ability as do more comprehensive experiments in artificially inoculated soil.

It is clear from a review of the literature that *P. arrhenomanes* and *P. graminicolum* attack many graminaceous hosts in both tropical and temperature climates. Doubtless, the finding of one or both of these species on wheat seedlings in the field in large wheat-growing countries, such as Australia and the U.S.A., where they have not hitherto been reported on wheat, merely awaits the search.

In England, by far the greater part of the wheat crop is winter wheat while, in those sections of the Canadian prairie provinces where the disease is common, only spring wheat is grown. This, together with the extreme differences in climate, makes it probable that the factors predisposing the plants to attack by the root parasites in the two countries differ not only in degree but also in kind. If, as has been contended (Carpenter, 1928, 1934; Cooke, 1934; Vanterpool, 1935), deficient or unbalanced nutrients may predispose plants to attack by *Pythium*, the use of fertilizers for a given soil type may alleviate the damage.

SUMMARY

The results of a comparative study of six species of *Pythium* pathogenic to wheat seedlings are presented. *P. arrhenomanes*, *P. volutum*, and *P. tardicrescens* n.sp., were found in both Canada and England; or, for *P. graminicolum* and *P. torulosum* [?] in England and not in Canada; and *P. aristosporum* n.sp., only in Canada. The English forms were obtained from Bucks, Cambs, Kent, Herts, Berks, and Lincs, and the Canadian forms from Saskatchewan. The similarity of the English form considered as a geographic strain of *P. torulosum* with the form reported on wheat in Italy by Petri is pointed out. Attention is drawn to the wide geographic distribution of species of *Pythium* on graminaceous hosts. By the method of isolation used, it was relatively easy to obtain one or more of these fungi either from wheat seedlings grown in potted soil collected at random from virtually any field with cereals in the rotation, or from

seedlings collected from the poorer looking parts of such fields. All of the parasitic species isolated belong to the group of the genus with lobulate sporangia. It is suggested that the many sphaerosporangial forms encountered may render the wheat seedlings more liable to attack by the pathogenic forms themselves. The use of fertilizers may prove beneficial.

Acknowledgements

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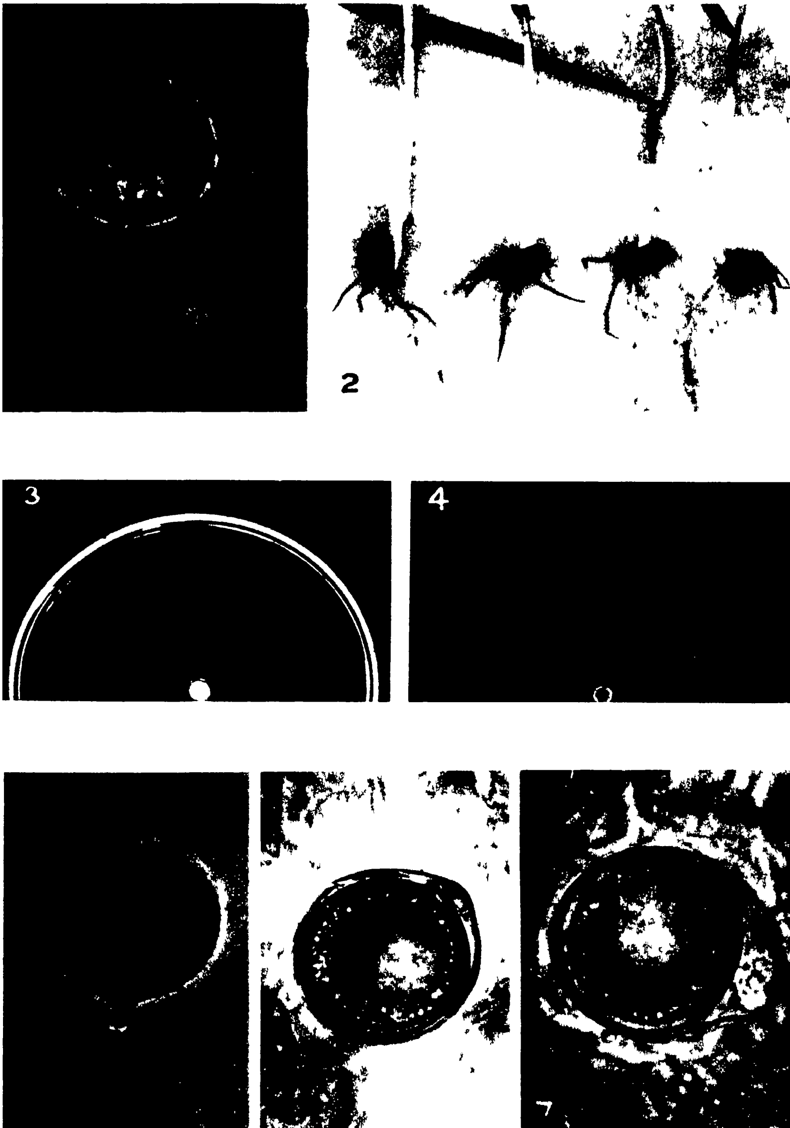

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EXPLANATION OF PLATE XXII

Fig. 1. *Pythium tardicrescens.* Oogonium with two diclinous antheridia arising from a single stalk, in a water culture containing sterile wheat root tips. × 900.

Fig. 2. Wheat seedlings, 4 weeks old, showing the characteristic root-tip lesioning following inoculation of sterilized soil with *P. tardicrescens.*

Fig. 3. A 2-day culture of *P. aristosporum* on carrot maize-meal agar, showing tendency for slight aerial development.

Fig. 4. A 2-day culture of *P. tardicrescens* on carrot maize-meal agar, showing the flat, radiate, non-lustrous growth.

Figs. 5, 6 and 7. *P. aristosporum* on water-agar containing wheat root tips. × 900. Fig. 5. A typical, mature, intercalary oospore, not filling the oogonial cavity. Fig. 6. An oospore with a protuberance on its wall. Fig. 7. A disorganized mass of protoplasm is to be seen between the oospore and the remains of the oogonial wall.

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FURTHER OBSERVATIONS OF THE INCIDENCE OF BLOTCHY RIPENING OF THE TOMATO

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(With 7 Text-figures)

CONTENTS

I. Introduction

Blotchy ripening of tomato fruits has been described and illustrated by Bewley & White (1926), who showed that this disorder, prevalent on plants grown in unmanured soil, is associated with a deficiency of nitrogen and potassium, especially the latter. The inverse relationship demonstrated between the percentage of blotchy fruit and the increments of potash and nitrogen added to the soil of plots deficient in these nutrients, provides strong evidence that the factors controlling the incidence of blotchy ripening are mainly nutritional.

Seaton & Gray (1936) consider that nutrient deficiency in relation to this disorder is "secondary and occasional" and advance the hypothesis that the primary cause of blotchy ripening is a "water-deficit" leading to sudden withdrawal of water from the fruit and mechanical disruption. They base this supposition on their interpretation of the morphological appearance in section of affected fruits, and do not present any experimental evidence to support their views. Robbins (1937) has recently

shown that a "water-deficit" leads not to blotchy ripening but to a separate physiological disorder—blossom end-rot.

II. THE EFFECT OF LIGHT ON THE INCIDENCE OF BLOTCHY RIPENING[1]

Table I (compiled from the Cheshunt Experimental Station *Annual Reports*) and Fig. 1, give the annual variations in proportion of blotchy fruit from plants grown during the period 1921–9 on plots from which nitrogen and potassium, respectively, were omitted continuously from the scheme of manuring. Fig. 1 shows that the annual fluctuations of one plot correspond with those of the other. It follows that there must be a third factor, affecting both plots, involved in the production of

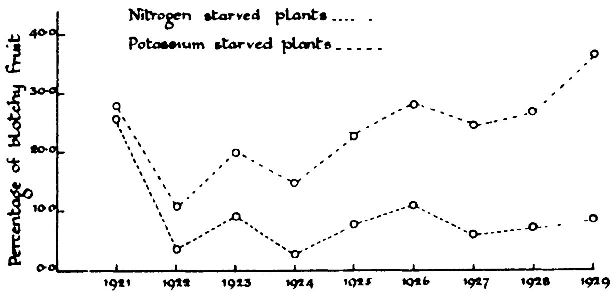

Fig. 1. Annual fluctuation in percentage of "blotchy" fruits of (*a*) potassium-starved plants, and (*b*) nitrogen-starved plants.

blotchy fruit. This factor is probably light, since the value of a correlation coefficient between the percentage of blotchy fruit on the nitrogen-starved plot and the mean daily number of hours of sunlight between 1 April and 31 August for the years 1922–7 inclusive is $-0{\cdot}803$ ($P=0{\cdot}03$). This negative association seems definite, and contrasts with the aberrant year 1921 when blotchy fruits were exceptionally prevalent in spite of an abnormally high number of hours of sunshine. Similar relationships, complicated by a rising trend in the proportion of blotchy fruit with time, are apparent in Fig. 1 for the fruit of the potassium-starved plants.

It is of interest to note that a definite positive correlation between blotchy fruit and high temperature cannot be traced. The correlation coefficient between daily maximal temperature and the values for blotchy fruit that are shown above to be correlated with light is $+0{\cdot}346$, a

[1] This and the following section are revised versions of parts of a thesis (unpublished) presented to the University of Cambridge in partial satisfaction of requirements for a Diploma in Horticultural Science.

statistically insignificant value. If the period between 1 June and 31 July when the influence of temperature on the water-relations of the fruit should be most marked is considered, the correlation coefficient is only +0·206. The correlation that may be traced between blotchy ripening and light confirms the view that this disorder is nutritional, whereas the low correlation coefficients between blotchy ripening and high temperature militate against the hypothesis of Seaton & Gray that blotchy ripening is primarily due to a water-deficit.

Table I

Annual variation in percentage of "blotchy" fruits

	Mean daily no. of hours of sunshine 1 April to 31 Aug.	Potassium-starved plants	Nitrogen-starved plants
1921	7·02	28·0	25·8
1922	6·25	10·8	3·7
1923	5·93	20·3	9·5
1924	6·22	15·1	2·9
1925	5·92	24·0	7·8
1926	5·47	28·5	11·3
1927	5·67	24·9	6·2
1928	5·95	26·8	7·3
1929	6·39	36·8	8·7

III. The differential effect of light on weight of fruit of potassium-starved plants

The weights of fruit from plants grown on plots, on which nitrogen and potassium, respectively, were omitted from the scheme of manuring over a period of 14 years, have been recorded in the Cheshunt Experimental Station *Annual Reports* from 1916 to 1929. These values have been discussed by Bewley (1929) who points out that the crop of the potassium-starved plants is relatively high in 1921, and suggests that the effect of light is equivalent to that of potassium in relation to nitrogen supply, so that in a sunny season nurserymen should manure with less potassium and more nitrogen. Bewley, referring to crop records from blocks with comparable manurial treatment over a period of many years, concludes "the yield per acre varies directly in relation to the total hours of bright sunshine 1 April to 30 September".

The nutrient deficiency experiments originally consisted of plots with duplicate treatments in adjacent houses. The treatments remained comparable until 1924, when the soil of one house was sterilized by steam, while the crop of the completely manured plants in the other shows, subsequently, the ageing effect usual in the continual cropping of glass-

house soils. Available data for 1916 are incomplete. Estimation of the error of the treatments is, therefore, facilitated by confining the discussion to the years 1917–23 inclusive.

Table II gives the crop in lb. per plant for the nitrogen-starved, potassium-starved, and completely manured plants. Since different varieties were used in the two houses the total weights of crop are not directly comparable. This difficulty may be overcome by calculating for each season the weight of crop of the nitrogen-starved and potassium-starved plants as a percentage of that of the completely manured plants. Fisher's analysis of variance has been performed on these relative values (columns 5, 7, 10 and 12 of Table II) and the results are given in Table III. Table III shows that the effects of season, treatment, and their interaction are significantly greater than the experimental error as estimated from duplication of the treatments. The outstanding feature of Table III is the high degree of significance of the interaction between treatment and season, and this effect outweighs that of treatment. This interaction must be due to a differential effect on potassium-starved and nitrogen-starved plants of some factor which is highly characteristic of seasonal

Table II

Weight of fruit in lb. per plant of completely manured plants (C.A.), *nitrogen-starved plants* (−N), *potassium-starved plants* (−K)

	Mean daily no. of hours of sunshine 1 April to 31 Aug.	House 1						House 2					
		C.A.	N	Relative	−K	Relative	C.A.	−N	Relative	−K	Relative
1917	6·27	5·11	5·60	109·6	4·65	91·0	5·64	6·08	107·8	5·18	91·8
1918	5·92	3·32	3·62	109·0	2·76	83·1	4·12	4·22	102·6	3·39	82·4
1919	6·28	5·57	5·98	107·4	4·95	88·9	5·86	6·34	108·3	5·46	93·3
1920	5·50	3·45	3·57	103·4	3·11	90·1	4·94	4·45	90·1	4·29	86·9
1921	7·02	4·08	4·27	104·7	4·65	114·0	5·84	5·54	94·8	5·74	98·2
1922	6·25	4·13	3·47	85·2	3·96	95·9	5·43	5·04	92·7	5·31	97·8
1923	5·93	5·09	4·69	92·1	4·51	88·6	5·40	4·70	87·1	4·69	86·8

Table III

Analysis of variance of annual fluctuation in crop of nitrogen-starved and potassium-starved plants

	Degrees of freedom	Variance	"Z"	1% point	5% point
Treatment	1	393·82	**0·709**	—	0·523
Season	6	102·59	**1·382**	1·091	—
Treatment × season	6	149·81	**0·890**	0·747	—
Remainder (error of duplication)	14	24·83	—	—	—

changes. Such factors are sunlight, temperature and rainfall. The effect of rainfall is discounted under glass, while the relative magnitude of the correlations with quality of fruit (p. 546) suggests that temperature does not have such a marked influence on fruiting as light.

Fig. 2 shows the annual variation in crop of the completely manured plants, together with the mean daily number of hours of sunlight from 1 April to 31 August, a period selected (White, 1925) as roughly corresponding with the period of fruiting of the tomato. The correlation coefficient between hours of sunlight and weight of crop is +0·438, a value which does not reach the conventional level of significance.

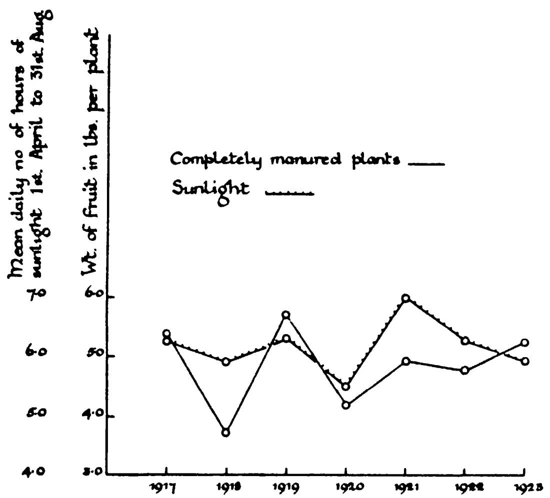

Fig. 2. Annual fluctuation in weight of fruit of completely manured plants together with the mean daily number of hours of sunlight from 1 April to 31 August.

Relative values of the crop of the potassium-starved and nitrogen-starved plants (Table II) are plotted in Figs. 3 and 4, in comparison with the daily number of hours of sunlight. The correlation coefficient between the relative values for the crop of the potassium-starved plants and light is +0·846, and this association must be considered definite since it would have occurred by chance in less than 2% of random cases. The crop of the nitrogen-starved plants falls throughout the period and it is necessary, therefore, to calculate correlation coefficients with time:

Nitrogen and light	...	$r_{12} = +0{\cdot}172$
Nitrogen and time	...	$r_{13} = -0{\cdot}922$
Light and time	...	$r_{23} = +0{\cdot}062$

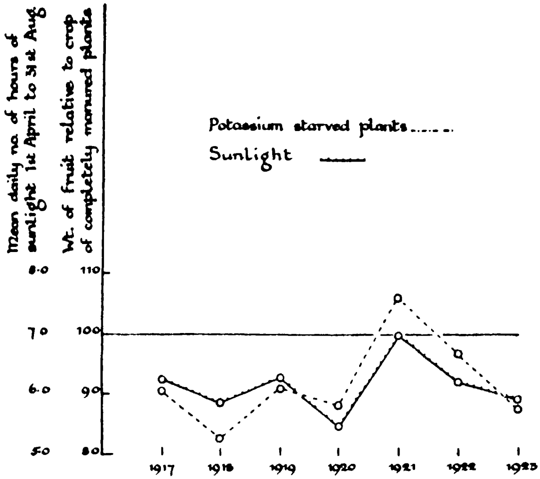

Fig. 3. Annual fluctuation in weight of fruit of potassium-starved plants relative to the crop of completely manured plants, together with the mean daily number of hours of sunlight from 1 April to 31 August.

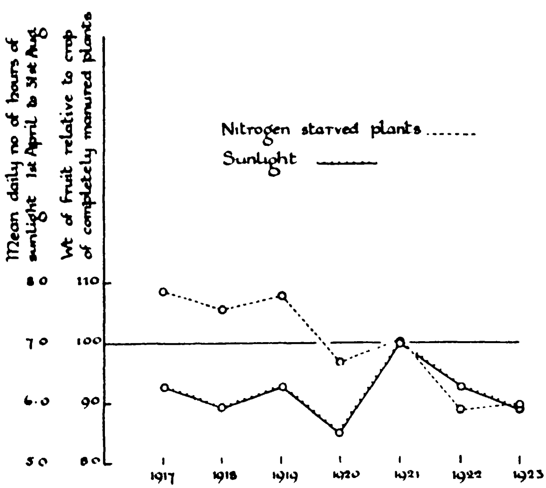

Fig. 4. Annual fluctuation in weight of fruit of nitrogen-starved plants relative to the crop of completely manured plants, together with the mean daily number of hours of sunlight from 1 April to 31 August.

The partial correlation coefficient between nitrogen and light, eliminating the effect of time, is +0·587. Although this correlation is higher than that characterizing the completely manured plants, it falls short of the level necessary to establish a definite association. It is concluded that the relationship between weight of crop of completely manured or nitrogen-starved plants and light is not of sufficient magnitude to be established by the data considered. In the potassium-starved plants, on the other hand, there is a close relationship between weight of crop and the level of the light factor.

Table IV

Effect, relative to potassium-starved plants, of increasing potassium supply

% of K_2O in fertilizer	Weight of fruit	"Blotchy" fruit
0	100·0	100·0
2½	102·5	45·1
5	118·3	31·6
7½	119·9	42·4
10	121·2	27·1
12½	124·3	24·8

Table IV summarizes the effect of increasing potassium supply on weight of fruit and on the proportion of blotchy fruit of potassium-starved plants. It is clear that a significant increase in crop and decrease in the proportion of blotchy fruit can be obtained *either by increase of potassium supply or by increase of light.*

IV. The effect of potassium on ripening

To elucidate the mode of effect of potassium in the production of blotchy fruit, reference may be made to the chemical changes taking place during ripening. These have been investigated by Sando (1920) who reaches the following conclusions: "The most striking change during ripening is that undergone by carbohydrates. In the first stage analysed it was noticed particularly that insoluble carbohydrates composed 52·1 % of the total carbohydrates present, while in the last stage, that of ripe fruit, soluble carbohydrates were in excess, amounting to 77·3 % of the total. Nearly all of the total sugar in the tomato fruit is apparently invert sugar and this increases from 25·56 % in the case of 14-day-old fruit to 48·32 % in ripe fruit, an increase of 89 %. Starch decreases during maturation from 15·84 to 2·65 %. The most marked decrease, as would be expected, is noticed during the period of transition from green to red," These observations suggest that failure of the blotchy areas of

fruit to ripen is associated with failure to convert starch into sugar. In order to test this hypothesis, portions of the carpel walls of normal fruits from the completely manured plants and blotchy fruits from the potassium-starved plants were cut out and grouped into samples of equal wet weight. Juice was extracted by pressure and equal volumes from both sets of samples were centrifuged. An ascending series of volumes of the juice obtained was mixed in a set of hard glass test-tubes with a descending series of volumes of a standard starch solution. Drops were extracted from time to time as indicators. After a few hours dilute iodine was added to each tube. *It at once became apparent that concentrations of starch that had been hydrolysed by the juice from the completely manured fruits were not being hydrolysed by the juice from the potassium-starved fruits.* Similar tests with "blotchy" and "normal" areas from the same fruits gave similar results. It is concluded that the unripened areas of the fruits of potassium-starved plants are characterized by low amylolytic activity. That blotchy ripening occurs in lesser degree in association with nitrogen starvation is of interest in view of the general belief that enzymes, including amylase, are of protein composition. Repetition of the experiment with substitution of "greenback" for "blotchy" fruits led to similar results.

V. Carbohydrate accumulation in leaves of potassium-starved plants

In order to obtain an indication of the effect of potassium on the translocation of carbohydrates from the leaves, changes in dry weight per unit area associated with potassium starvation were compared with corresponding changes with full nutrient supply and with nitrogen starvation. Since it was impracticable to use whole leaves the area of corresponding leaflets was estimated by enclosing the leaves between thin panes of glass, drawing an illuminated outline on tracing paper and tracing the areas with a planimeter. The leaves were subsequently dried at 100° C. and weighed. The work had to be limited to comparison of a single plant from each treatment. Care was taken to select a normal completely manured plant, and plants with characteristic though moderate symptoms of nitrogen and potassium starvation. The terminal pair of leaflets of every leaf on the plants was used. The results are shown in Table V. The dry weight per unit area of the nitrogen-starved plant is consistently high and the effect is visible even in the youngest leaf. The dry weight per unit area of the completely manured and potassium-starved plants are plotted in Figs. 5 and 6. The dry weight per unit area

of the completely manured plant increases from the youngest to the sixth leaf from the growing point, but remains relatively constant or

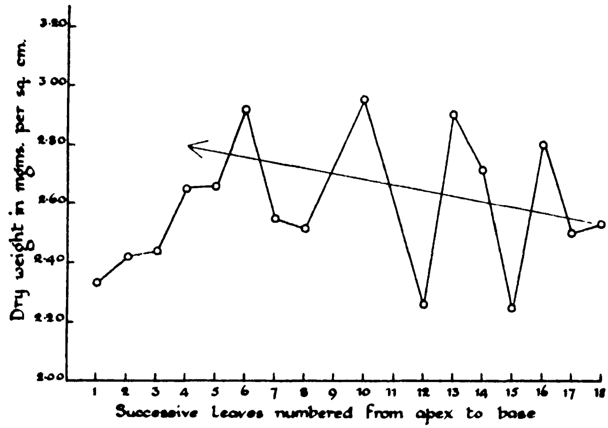

Fig. 5. Dry weight per unit area in mg. per sq. cm. of corresponding leaflets of successive leaves, numbered from apex to base, of a completely manured plant.

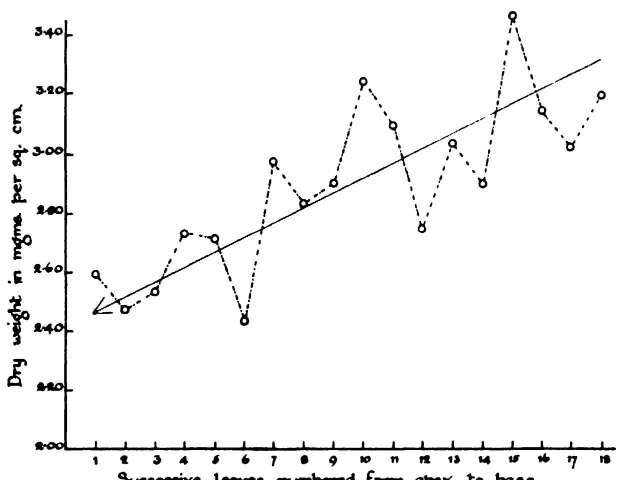

Fig. 6. Dry weight per unit area in mg. per sq. cm. of corresponding leaflets of successive leaves, numbered from apex to base of a potassium-starved plant.

falls slightly in older leaves. The dry weight per unit area of the leaflets of the potassium-starved plant *continues to increase from the youngest to the oldest on the plant.*

Table V

Dry weight (mg. per sq. cm.) of corresponding leaflets from every leaf (numbered from apex to base) of a typical plant from each treatment

Leaf no.	Completely manured		Nitrogen-starved		Potassium-starved	
1	2·29	2·33	2·72	2·74	2·61	2·59
	2·37		2·77		2·57	
2	2·33	2·42	2·91	2·94	2·57	2·47
	2·51		2·97		2·37	
3	2·48	2·44	2·99	3·01	2·53	2·53
	2·39		3·04		2·54	
4	2·67	2·65	2·97	2·99	2·86	2·73
	2·62		3·02		2·60	
5	2·70	2·66	3·28	2·27	2·75	2·71
	2·62		3·25		2·66	
6	3·00	2·92	3·14	3·18	2·34	2·43
	2·84		3·22		2·52	
7	2·65	2·55	2·98	2·98	3·01	2·97
	2·44		2·97		2·93	
8	2·44	2·51	3·12	3·01	2·87	2·83
	2·58		2·91		2·79	
9	2·95	2·95	2·41	2·49	2·88	2·90
	2·96		2·58		2·92	
10	2·30	2·26	2·61	2·58	3·22	3·24
	2·22		2·54		3·26	
11	2·86	2·90	2·67	2·67	3·12	3·09
	2·94				3·05	
12	2·75	2·71	2·67	2·66	2·75	2·74
	2·66		2·65		2·73	
13	2·22	2·24	2·58	2·65	2·89	3·03
	2·26		2·72		3·18	
14	2·81	2·80	—	—	2·89	2·89
	2 78				2·90	
15	2·32	2·50	—	—	3·80	3·46
	2·69				3·11	
16	2·71	2·53	—	—	3·10	3·14
	2·35				3·18	
17	—	—	—	—	3·15	3·02
					2·88	
18	—	—	—	—	3·31	3·19
					3·07	

To demonstrate further that severity of potassium starvation affects carbohydrate accumulation in the leaves, four plants were selected from the no-potassium plot. These plants were ranged in order according to severity of starvation symptoms. Differences between the two plants with most severe starvation symptoms were slight and have been averaged in plotting the results. These values, shown in Table VI and Fig. 7, confirm that increasing severity of potassium starvation is associated with progressive increase in level of dry weight per unit area.

Table VI

Dry weight (mg. per sq. cm.) of corresponding leaflets of leaves (numbered from apex to base) of plants showing (a) slight, (b) moderate, (c) and (d) severe symptoms of potassium starvation

	(a)		(b)		(c)		(d)	
1	3·57	3·62	4·06	3·87	4·33	4·50	4·12	4·12
	3·67		3·68		4·69		4·12	
2	3·62	3·54	4·27	4·34	4·66	5·02	4·88	4·55
	3·46		4·40		5·38		4·22	
3	3·13	3·19	4·19	4·16	4·86	5·05	5·11	5·10
	3·25		4·12		5·23		5·09	
4	3·06	3·13	3·90	3·91	4·36	4·34	5·00	4·95
	3·19		3·92		4·31		4·89	
5	2·81	2·81	3·61	3·82	3·35	3·50	4·23	4·15
	—		4·02		3·64		4·06	
6	3·30	3·33	4·09	4·09	4·26	3·92	5·17	5·11
	3·35		—		3·58		5·04	

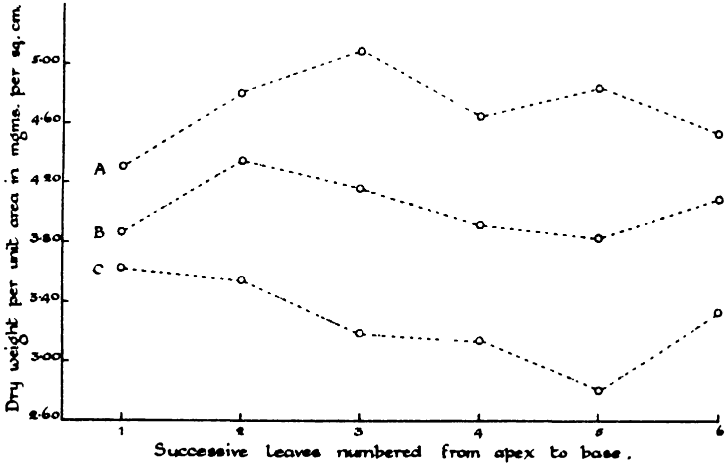

Fig. 7. Dry weight per unit area in mg. per sq. cm. of corresponding leaflets of successive leaves numbered from apex to base of plants showing (A) slight, (B) moderate, and (C) severe symptoms of potassium starvation.

VI. The histology of blotchy fruit

A histological examination of the affected tissues of blotchy fruit was carried out (White, 1925) and the following conclusions were reached:

(1) Blotchy ripening is not invariably associated with structural disorganization of the tissues, which appears to follow rather than precede failure to ripen. Extensive plugging may occur, especially in

the cells of the phloem, but also, occasionally, in the woody elements, without obvious structural damage to either of these tissues or adjacent parenchyma.

(2) The membranous lining of the cavities adjacent to necrotic bundles is derived in part from disorganized parenchyma and in part from the phloem, the cross-walls of these cells disintegrating and the longitudinal walls thickening extensively.

These observations are not in complete agreement with those of Seaton & Gray (1936) who claim that the vascular bundles of blotchy fruits are unaffected, but appear necrotic because of the draping around them of the broken-down cell debris of adjacent parenchymatous tissue.

VII. Discussion

Increase in number of hours of sunlight is associated with decrease in percentage of blotchy fruit despite a tendency to increase in weight of crop, which should be associated with increased consumption of nutrients and, therefore, with greater intensity of potassium and nitrogen starvation. This suggests that blotchy ripening is not due directly to lack of potassium or nitrogen but to metabolic changes that are counteracted by increase of light.

The increase in weight of crop associated with increase of sunlight is statistically significant, under the experimental conditions, only in the case of the potassium-starved plants. Since previously observed effects of potassium deficiency on fruiting—notably acceleration of blossoming and prolongation of the ripening period (White, 1938)—are associated also with low carbohydrate level, independently of potassium supply, it is reasonable to attribute the beneficial effect of increase of light on weight of fruit and proportion of blotchy fruit of potassium-starved plants to increase in carbohydrate level.

Increasing severity of potassium starvation is association with progressive increase in level of dry weight per unit area of the leaves. This accumulation of surplus carbohydrate in the leaves of potassium-starved plants, in conjunction with the similarity between the effects of potassium deficiency on fruiting and those of plants with relatively low carbohydrate level, favours the view that translocation of carbohydrates is impaired in potassium-starved plants. In support of this view may be cited the experiments of Phillips *et al.* (1934) who show that the starch and dextrin content of potassium-deficient tomato plants is high in the leaves but low in the stems.

The juice of the affected areas of blotchy fruit is characterized by low amylolytic activity; moreover, the cell walls of the phloem of the vascular bundles of affected areas of blotchy fruits may be characterized by extensive thickening. It is now generally accepted that the phloem is the sugar-conducting tissue of plants, and it may well be that sugars are being condensed to cellulose in the vascular bundles of blotchy fruits of potassium-starved plants, instead of participating in the normal processes of ripening.

These observations are conformable with the view that blotchy ripening is symptomatic of deranged carbohydrate metabolism, the fruits of potassium-starved plants being characterized by abnormal carbohydrate changes, while translocation of carbohydrates from the leaves to the fruits is impaired, possibly owing to the occurrence of similar changes in the leaves. It may well be that these changes are accompanied by derangement of the water relations, leading to the association of blotchy ripening with disruption of fruit tissues, since the prevalence of Leaf Scorch on potassium-starved plants (Bewley & White, 1926) suggests lack of balance between water supply and water loss.

VIII. Summary

1. The annual fluctuation in percentage of fruit affected with "blotchy ripening" on potassium-deficient and nitrogen-deficient plots at the Cheshunt Experimental Station is significantly negatively correlated with the mean daily number of hours of bright sunshine between 1 April and 31 August.
2. The weight of fruit of potassium-deficient plants is raised and the percentage of blotchy fruit reduced, either by increase of potassium supply or by increase of light. This beneficial effect of increase of light on the crop of potassium-deficient plants is of much greater magnitude than any corresponding effect on the crop of nitrogen-deficient or completely manured plants.
3. The juice of the blotchy areas of potassium-starved fruits has diminished capacity for starch hydrolysis.
4. Increasing severity of potassium starvation is associated with progressive increase in level of dry weight per unit area of the leaves.
5. The influence of potassium on the production of blotchy fruit is briefly discussed.

The author is indebted to Dr W. F. Bewley for permission to publish this paper.

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PHYSIOLOGICAL RELATIONSHIPS BETWEEN INSECTS AND THEIR HOST PLANTS

I. THE EFFECT OF THE CHEMICAL COMPOSITION OF THE PLANT ON REPRODUCTION AND PRODUCTION OF WINGED FORMS IN *BREVICORYNE BRASSICAE* L. (APHIDIDAE)

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(With 4 Text-figures)

CONTENTS

I. Introduction

Much work has been carried out upon the biological relationships existing between insects and their host plants (Davidson, 1925; Painter, 1936; Trouvelot *et al.*, 1933). It has been established that different species and varieties of host plant, manurial conditions, etc., may have great effects upon the rate of reproduction, rate of growth and longevity of the insects feeding upon them, but little study has been made of the factors in the plant that bring about these effects, i.e. whether they are due to variations in the general nutritive level of the plant, presence or absence of specific substances giving a suitable or unsuitable taste, presence of poisons, lethal proteins, absence of vitamins or salts. Painter gives an interesting and extensive discussion of the types of relationship which are likely to exist between insects and plants, at the same time emphasizing the paucity of exact chemical data in existence.

The insect chiefly used in the present work is the cabbage aphis, *Brevicoryne brassicae* L. It has many advantages for this type of work; many generations can be bred in a short time and small space and it has a wide variety of host plants (cf. Petherbridge & Mellor, 1936). The effect of the host on the production of apterous, alate and sexual forms can be studied and its common food-plant, cabbage, can easily be grown. It has the marked disadvantage, however, that its principal food, the contents of the phloem tubes, cannot be extracted for analysis. Therefore, it has been necessary to analyse the leaf lamina and to use its chemical composition as the closest approximation obtainable at present. On account of the size of the aphis, it is very difficult to obtain adequate quantities of a uniform age for chemical analysis. Davidson (1925) suggested that the manurial treatment of the plant has a definite effect in increasing the percentage dry weight of *Aphis rumicis* L. and, therefore, the absolute nitrogen content. However, there is clearly something incorrect in his table since he records a dry weight of 0·215 g. per individual aphis, an improbable figure for this species.

II. METHODS OF ANALYSIS

The plant material was preserved for analysis in the following manner: the leaves were divided into two portions lengthwise, discarding the midrib, one half of the sample was put into boiling 95% alcohol, boiled for a few minutes and preserved in this; the other half was dried at 60° C. to constant weight. Analyses of soluble sugars and starch were carried out on the material preserved in alcohol, and the dried material was used for dry weight determinations and estimation of nitrogen.

Nitrogen. The unmodified Kjeldahl method was used. Non-protein nitrogen was extracted by grinding up the material in 2·5% trichloracetic acid in 0·02% phenol for 20 min. and filtering. The insoluble nitrogen of the residue is termed protein nitrogen (Richards & Templeman, 1936).

Soluble sugars. The soluble sugars were extracted by boiling the material in 80% alcohol for 8 hr., filtering, and evaporating off the alcohol under reduced pressure at 35° C., the residue being diluted with distilled water and cleared with a few drops of dibasic lead acetate as prepared by Van Plank (1936). Excess lead was removed with 3·1% disodium hydrogen phosphate. An aliquot of the cleared solution was acidified with hydrochloric acid to 0·4% and heated on a water-bath at 70° C. for 15 min. to hydrolyse any sucrose present. The solution was then

neutralized and the sugars estimated by the method of Shaffer & Somogyi (1933).

Starch. The starch was extracted from the sugar free residues by boiling them for 15 min. in 95% alcohol to which 1 ml. of concentrated hydrochloric acid per 100 ml. had been added (Hanes, 1936). The acid alcohol was filtered off and the starch extracted by boiling the treated material in 20 ml. of water for 20 min. Two extractions sufficed. The starch was hydrolysed in 2·5% hydrochloric acid for 2 hr. on a boiling water-bath. The amount of sugar present was estimated by the Shaffer-Somogyi method after neutralization.

III. The influence of the chemical composition of the host plant on reproduction

Two experiments were carried out which enable the effects of the nitrogen and carbohydrate contents of the host plant on reproduction to be studied. One experiment was expressly designed for this study while the other, designed for another purpose, gave results of interest on these points. A wide variation in the chemical composition of the host plant used (Sutton's cabbage, variety Tender and True) was obtained by growing one set of six plants under cellophane cages and another set of six plants under cellophane cages covered with two layers of Courtauld's black art silk voile which cut out 80% of the light. The voile covers were placed on the cages 3 days before the insects were placed on the plants. On 25 August 1936 each plant was infested with three young apterous aphides born of winged parents. Reproduction commenced on 27 August and the experiment was terminated on 5 September. Table I shows the number of aphides produced on each plant.

Table I

Number of young produced by three apterae in 10 *days under light and dark conditions of illumination of the host plant*

Cage ...	1	2	3	4	5	6	Mean
Light	58	51	77	77	59	84	68
Dark	4	1	15	26	2	23	12

The difference in the rate of reproduction of the aphides fed on plants grown under normal light conditions and those grown under subnormal conditions of light is clearly significant. This experiment is referred to below as Exp. 1. In view of the work of Shull (1930) on the influence of light on the production of winged forms, it was suspected that the direct influence of light upon the aphides themselves might

have affected the rate of reproduction. This however did not prove to be so, as aphides placed in smaller cages under the above conditions of light on the petioles of cabbage leaves, the laminae of which were exposed to full light, did not show any significant difference in their rate of reproduction. The mean number of young produced by six sets of three apterous aphides under normal light conditions was ten, while under subnormal light conditions it was fourteen.

The second experiment consisted of six plants under cellophane and six under perforated zinc covered with cellophane. The plants were divided into six blocks, each block consisting of one pair of dissimilar cages. The cellophane cages in each block were infested, at random, with a number of aphides varying from one to six and the corresponding zinc cage of each pair with double the number. This experiment was primarily designed to study the effect of the chemical composition of the host plant on the production of alatae, and Davidson (1925) has shown that the rate of reproduction of *Aphis rumicis* on beans was about halved by growing them under perforated zinc cages. In this experiment the plants growing under the zinc cages were therefore infested with twice as many individuals as those growing under cellophane cages in order to eliminate as far as possible any effects of overcrowding on the production of alatae. The plants were grown in a shady insectary and were infested on 21 July 1936 with nearly full grown apterae born of apterous parents. Reproduction was occurring on all plants on 24 July and the experiment was terminated on 14 August. All the parent aphides were alive on 27 July and each had produced about ten young; there was no difference between the rates of reproduction in the two sets of cages at this date but marked differences had appeared when the experiment was terminated. The mean number of young descended from one female under light conditions was 445 and under the darker conditions was 160. The variations about the mean were great in each case but the difference is significant when examined by the t test, $P = 0{\cdot}01$. This experiment is referred to below as Exp. 2.

The plants in Exp. 1 were analysed for total nitrogen, soluble sugars and total water-soluble carbohydrate (soluble sugars + starch) on a wet weight basis. In Exp. 2 enough material was available to fractionate the total nitrogen into protein nitrogen and non-protein nitrogen. Fig. 1 shows the relationship between percentage total nitrogen and the number of young produced by three apterae in Exp. 1. It shows clearly that, in general, the higher the percentage total nitrogen the greater is the number of young produced. The correlation coefficient between the two

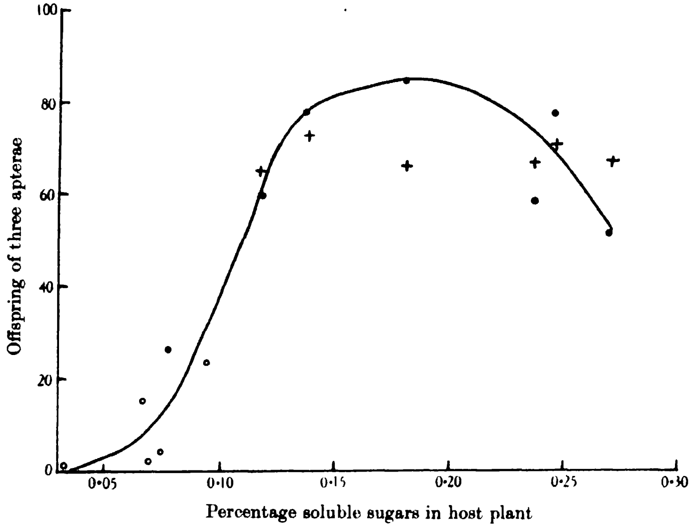

Fig. 2. The relation between reproduction of *Brevicoryne brassicae* and percentage soluble sugars in the host plant: Exp. 1. • Plants grown in normal conditions of light. o Plants grown in subnormal conditions of light. + + for explanation see text.

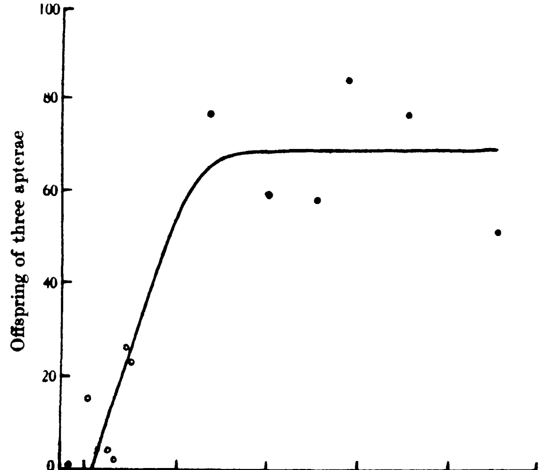

Fig. 3. The relation between reproduction of *Brevicoryne brassicae* and percentage total water-soluble carbohydrate in the host plant: Exp. 1. • Plants grown in normal conditions of light. o Plants grown in subnormal conditions of light.

show that the apparent depressant effect of excess soluble sugars is fortuitous. The resultant figures are indicated in Fig. 2 by crosses. No clear relationship was found between the percentage soluble sugar or percentage total carbohydrate and reproduction in Exp. 2. The correlation coefficient between percentage soluble sugars and reproduction is 0·4070 and is not significant.

The carbohydrate content of the plant might play a critical part in determining the rate of reproduction during the short dull days of early spring and late autumn in some species of aphides. Watson (1936) finds it necessary to illuminate with artificial light for 2 hr. daily the radish and turnip plants on which she rears *Myzus persicae* from November to January in order to keep the insects alive. The aphides are reared in a heated greenhouse, therefore temperature is not a limiting factor. It is possible that, under normal conditions of light at these times of the year, the carbohydrate content of the plant falls below the minimum requirements of the insects and the extra hours of illumination serve to increase it above that minimum. *B. brassicae*, however, reproduces slowly during the winter if the temperature is suitable (Petherbridge & Mellor, 1936).

The present data do not show whether percentage soluble sugars or percentage total water-soluble carbohydrate is the significant variable in the relationship between the aphides and the carbohydrates of the plant. Since there is a highly significant correlation between the percentage soluble sugars and the percentage total water-soluble carbohydrate, a definite decision cannot now be made upon the point. In Exps. 1 and 2 the correlation coefficients between percentage soluble sugars and percentage total water-soluble carbohydrate are 0·9607, $P < 0{\cdot}01$ and 0·9794, $P < 0{\cdot}01$ respectively. Davidson (1923) has examined sections of plants infested by aphides and finds that they feed upon the contents of the phloem elements in the vascular bundles, but that cells of the epidermis, cortex, mesophyll and xylem may be tapped for nourishment, especially when the host is heavily infested or in poor condition. Thus, whether the soluble sugar content or the total water-soluble carbohydrate is the significant variable may depend on the density of the population of aphides on the plant. When this is low the insects feed principally on the contents of the phloem in which starch is absent and, since the concentration of sugars in the phloem is a function of the concentration of sugars in the leaf, it would appear that the latter would be a valid estimation since it is, at present, impossible to analyse the contents of the phloem.

Since the aphides feed principally on a food which is not known to

contain starch, the presence of a starch hydrolysing enzyme in the salivary glands (Davidson, 1923) may appear somewhat peculiar. Its usefulness may, perhaps, be dependent on circumstances. When the population density on the plant is low, and the aphides are feeding on the phloem contents, its value is nil. When, however, the population density is high and the aphides are feeding on the contents of starch-containing cells, the injection of extra amylase may serve to hasten the hydrolysis of the starch into soluble degradation products.

No data have yet been obtained on the effect of the chemical composition of the host plant on the rate of growth of aphides but Table III gives some data on the weights of larvae of *Pieris brassicae* fed on cabbage leaves grown under the experimental conditions detailed above for Exp. 1. The larvae had a mean weight of 0·2 mg. when the experiment commenced; ten individuals were reared under each of the two conditions of nutrition and were weighed individually at intervals. On and after the tenth day the mean weights of the two groups differ significantly as do those of the sixth day. It is thought that the similar mean weights for the two batches on the eighth day are due to the fact that the larvae grown on normal food were preparing to moult when weighed but the others were still feeding so that the former lot were weighed with empty alimentary canals. The larvae fed on normal food moulted about 12 hr. before the others, ceased to feed 2 days before and pupated 3 days before the others.

Table III

Effect of host plant grown under (A) *normal light conditions,* (B) *sub-normal light conditions, on increase in weight in mg. of* Pieris brassicae *larvae*

Days	2	6	8	10	13	17	21	23	25	Pupation
A	0·8	4·4	6·7	29	94	319	618	613		452
B	0·8	3·4	6·5	16	46	161	415	504	562	389
Ratio B : A	1 : 1·0	1 : 1·3	1 : 1	1 : 1·8	1 : 2	1 : 2	1 : 1·5	1 : 1·2		1 : 1·2

The ratio of the weight of the larvae fed on food grown under sub-normal light conditions to that of larvae fed on food grown under normal light conditions shows that the latter grew much more rapidly than the former during the first half of the growth period so that they became twice as heavy but, during the last instar (17th day and onwards), the former were able to make up some of the lost ground and, at pupation, weighed but little less. In spite of the great difference in weight of the larvae at the commencement of the last instar, no significant difference was noted in the width of the shed head capsules.

IV. The effect of the insect on the chemical composition of its host plant

A number of small plants were caged under cellophane and infested on 22 August 1936 with varying numbers of apterous aphides. Reproduction was allowed to continue until some of the plants were thoroughly infested. The experiment terminated on 14 September. To obtain an index of the intensity of infestation, the total number of aphides present on the plant was divided by the weight of the leaf laminae in grammes. The index of infestation so obtained is called the population density and, in this experiment, it varied from 109 to 413. Unfortunately, the plants used in this experiment were somewhat varied in size to begin with, and it is thought that the varying chemical composition of the plants associated with varying vigour of growth has obscured the results obtained. This experiment is referred to below as Exp. 3. No significant correlation was found between population density and either plant weight, percentage dry weight, percentage soluble sugars or percentage total carbohydrate. In another set of data (not yet analysed completely) a very definite relationship has been found between population density and the wet and dry yields of leaves and percentage dry weight. There is, however, in Exp. 3, a negative correlation between population density and percentage total nitrogen, $= -0{\cdot}7430$, $P > 0{\cdot}02$ but $P < 0{\cdot}05$. The percentage total nitrogen was analysed into percentage protein nitrogen and percentage non-protein nitrogen but no significant correlations were found. The results, however, suggest that relationships may exist, but discussion of them must be postponed until further data are available.

V. The effect of the chemical composition of the host plant on the production of alatae

Various explanations have been put forward to account for the appearance of winged aphides under different conditions. It has been established that a higher proportion of alatae occurs on over-crowded plants than on sparsely infested plants (Reinhardt, 1927). Shinji (1918) produced some evidence that solutions of various salts and organic substances, especially sugar, when introduced into infested rose cuttings would cause variations in the percentage of alatae ultimately produced on the shoots. However, confirmatory evidence was not obtained by several later workers. Ackerman (1926), Reinhardt (1927), and Wadley (1931) found that starvation of either adults or first or second instar nymphs, according to the species of aphis, would induce the formation of

alatae. Shull (1930) studied this problem in great detail with reference to light, attempting at the same time to eliminate any effect of the chemical composition of the plant on the formation of alatae. He claimed that he was able to demonstrate that varying times of exposure to light and varying intensities of light caused a variation in the percentage of alatae produced, and that the effect was directly due to light. However, he was forced to conclude in one experiment that it was probably the chemical composition of the plants which caused the production of alatae. Shull did not analyse his plants and so his claim that the chemical composition of the plant did not affect his results must be regarded with caution until his elaborate experiments are repeated together with analyses of the plants. Rivnay (1937) has recently produced evidence to show that, in *Toxoptera aurantii* Boy., lack of water in the shoot on which the insects are feeding may bring about the production of alatae, but his sweeping assertions that "temperature does not affect wing development in Aphids" and that "all such factors as light, temperature, crowding, humidity, precipitation, growth of plant, etc., exert, directly or indirectly, an influence on the water balance in the body of the aphid which in turn causes wing development" cannot be admitted until much more work is done on more than one species of aphis. Most writers are agreed that it is unsuitable conditions in the host plants which bring about the formation of alatae and so it is quite possible that, according to circumstances, different factors within the host plant may have this effect. Evidence will be adduced below to show that in *B. brassicãe* the protein content of the host plant had very definite influence on the production of alatae.

In Exp. 3 a varying percentage of alatae occurred on the different plants; the correlation coefficient between population density and percentage alatae was not quite significant, $r = 0{\cdot}6697$, $P > 0{\cdot}05$ but $P < 0{\cdot}10$, but the correlation coefficient between percentage total nitrogen and percentage alatae was much higher $= -0{\cdot}8199$, $P > 0{\cdot}01$ but $P < 0{\cdot}02$. As figures are available for the percentage protein nitrogen and percentage non-protein nitrogen, it is possible to examine which of these fractions is the more significant in determining the production of alatae. The correlation coefficient between percentage alatae and percentage protein nitrogen is $-0{\cdot}8825$, $P < 0{\cdot}01$, while that between percentage alatae and percentage non-protein nitrogen is $0{\cdot}6266$, $P = 0{\cdot}10$. There is, therefore, a highly significant negative correlation between the production of alatae and the protein content of the plant, but the correlation between percentage alatae and percentage non-protein nitrogen is not

significant. Fig. 4 shows that in Exp. 2 there is clearly a relationship between percentage alatae and both percentage total nitrogen and percentage protein nitrogen. No relationship is to be found between percentage alatae and percentage non-protein nitrogen in this experiment. Table IV gives the data obtained from Exp. 2 on this point.

With regard to the relationship between percentage alatae and the carbohydrate content of the plant, none was found in Exp. 3 but in Exp. 2, Table IV shows that there is an increasing percentage of alatae as the amount of soluble sugars and of total water-soluble carbohydrate diminishes. In this experiment, it is not possible to differentiate between

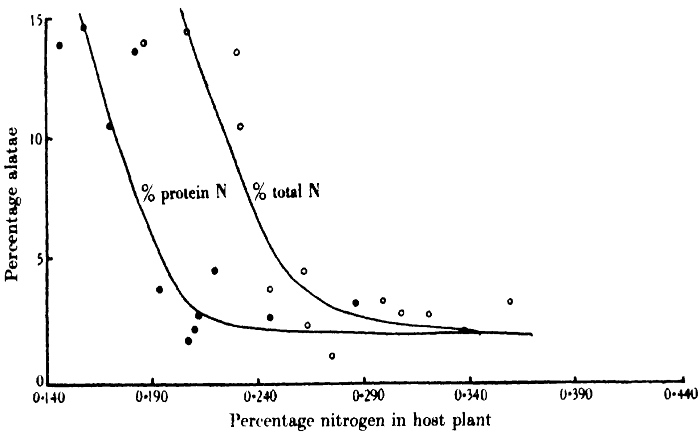

Fig. 4. The relation between the formation of winged forms of *Brevicoryne brassicae* and the percentage of nitrogen in the host plant: Exp. 2.

Table IV

The effect of the chemical composition of the host plant on the production of alatae

Exp. 2.

% Alatae	14·8	14·3	13·9	10·7	4·8	4·0	3·4	2·9	2·2	2·1
% Protein N	0·159	0·146	0·184	0·171	0·221	0·195	0·287	0·248	0·339	0·209
% Non-protein N	0·052	0·042	0·049	0·064	0·043	0·052	0·075	0·062	0·092	0·067
% Soluble sugars	0·089	0·071	0·075	0·093	0·111	—	0·235	0·137	0·142	0·091
% Total water-soluble carbohydrates	0·237	0·232	0·250	0·262	0·303	—	0·586	0·445	0·368	0·263
% Water	92·5	93·0	92·1	92·4	92·4	92·9	89·4	91·0	90·7	92·1

Exp. 3.

% Alatae	26·1	23·4	22·9	18·5	12·0	9·1	3·4	1·6
% Total N	0·304	0·285	0·283	0·293	0·310	0·335	0·333	4·14
% Protein	0·173	0·166	0·194	0·211	0·209	0·250	0·254	0·336
% Water	90·9	92·1	91·5	92·3	91·3	92·0	91·8	88·6

Table V

The effect of carbohydrate on the production of alatae

Treatment...	Light					Dark					Very dark				
Cage ...	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean
% Alatae	2·9	7·4	4·0	4·1	4·6	2·5	6·1	4·0	4·5	4·3	0	3·2	0	9·3	3·1
% Soluble	0·243	0·261	0·192	0·257	0·238	0·221	0·189	0·238	0·177	0·206	0·092	0·075	0·077	0·149	0·098
% Total water-soluble carbohydrates	0·632	0·719	0·454	0·611	0·604	0·537	0·492	0·571	0·418	0·505	0·247	0·151	0·233	0·337	0·242
Water	87·1	90·8	90·7	86·5	88·8	92·9	93·5	92·0	92·0	92·6	93·2	93·6	94·1	93·1	93·1

the effect of protein and carbohydrate on the production of alatae. An experiment was set up to test whether the carbohydrate content of the plant had any effect on the production of alatae. A set of twelve cabbage plants, selected for uniformity of size, were divided into four blocks of three plants and subjected to the following treatments of light intensity, (*a*) cellophane cages, (*b*) cellophane cages covered with one layer of black voile, (*c*) cellophane cages covered with two layers of black voile. The results are given in Table V and show conclusively that wide variations in the carbohydrate content of the plant do not affect the production of alatae.

Tables IV and V also show that the percentage of water in the plants had no effect on the production of winged forms in *B. brassicae*. It is, of course, possible that the critical factor in the plant that brings about wing formation will vary from species to species according to the host plant and climatic conditions; thus in the xerophytic conditions under which *Toxoptera aurantii* lives, water might well be a critical factor. The problem as to what factors within the aphis induce the production of wings is as yet unstudied except for the suggestion of Rivnay that a low water content will bring it about and some speculations by Ackerman (1926) and Shull (1930) who postulate the presence of a substance which by concentration or change into another substance respectively determined whether apterae or alatae will develop. A study of the relation between ductless glands, such as the corpus allatum, and wing production might produce some interesting results.

No data on the effect of the quality of the food on the rate of feeding have been obtained and the work of Hamilton (1935) shows that to obtain any accurate data of this kind would be very difficult. Some information bearing on this factor has, however, been obtained with larva of *Pieris brassicae*. Half-grown larvae were fed on normal cabbage leaves

and on leaves kept under a double thickness of black voile for 24 hr. The weights of faeces voided by each of five larvae feeding on the two types of leaves for 18 hr. was recorded. From the normal leaves an average of 0·081 g. was produced; from those kept under dark conditions an average of 0·171 g. The mean increments in growth of the batches of larvae during the experiment were very similar, being 37 and 39%, so that the difference in the mean weights of the faeces is not due to the larvae which had fed on normal food utilizing more of it than those fed on leaves kept under dark conditions. The conclusion drawn from the data is that the larvae feeding on the food of poor quality (especially in carbohydrates) actually consumed much more than those feeding on normal leaves.

When the percentage protein nitrogen in the plant reaches a low level, there is a rapid increase in the percentage alatae of *Brevicoryne brassicae* produced. Whether this increase is due to the quantity of nitrogenous food falling below a definite required amount necessary to produce apterae or to a change in the quality of the protein is not known. It is hoped that this aspect of the problem will be studied later.

VI. SUMMARY

It is shown that under late summer conditions of light the rate of reproduction of the aphis, *Brevicoryne brassicae*, is positively correlated with the nitrogen content of the host plant and, in particular, with the protein content. The formation of winged forms is negatively correlated with the same factors.

The chemical composition of the plant affects the rate of growth, length of larval period and final pupal weights of *Pieris brassicae*. It also influences the amount of food eaten.

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SHEEP BLOW-FLY INVESTIGATIONS

VII. OBSERVATIONS ON THE DEVELOPMENT OF EGGS AND OVIPOSITION IN THE SHEEP BLOW-FLY, *LUCILIA SERICATA* MG.

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Introduction

Although *Lucilia sericata* Mg., the sheep maggot-fly, has been the object of intensive study by insect physiologists during recent years, comparatively little attention has been paid to the adult stage. This is surprising, since it is the adult female's habit of ovipositing on live sheep that is primarily responsible for the enormous damage caused by this species. Accordingly, investigations have been carried out on blow-flies with reference to the development of eggs and oviposition. The previous work need not be reviewed here in detail since Evans (1936) has recently summarized our knowledge of the physiology of *L. sericata*. Whereas the males become sexually mature within a few hours after emergence, the females require several days before the ovaries are fully developed; furthermore, this only occurs if the diet includes meat. On the other hand, fertilization by the male is not necessary for the development of the ovaries or for oviposition; my experience has been, however, that unfertilized females do not oviposit readily. When a female oviposits, the ovaries are as a rule completely emptied of eggs, and a fresh batch of eggs develops later. The number of eggs in a batch depends on the size of the fly and varies from about 80 to 170.

Experimental

Effect of temperature on the development of the ovaries

For these investigations the flies were kept in jam jars half-filled with sand and placed in incubators; to prevent the flies escaping, their wings were clipped and the jars were covered with gauze. The flies were fed on sugar, meat and water, which were renewed daily; the humidity was kept high by the drinking water in the jars and by water exposed in the incubator. Provided that only a small piece of meat was placed in the

jar, the flies remained healthy[1] under these conditions, and, if fertilized, oviposited at regular intervals. For studying the initial development of the ovaries, female flies were taken as soon as they emerged from the pupal cases and transferred to jars in batches of about eight. The growth of the ovaries was followed by dissecting flies at intervals. In order to investigate the development of eggs subsequent to the first oviposition, it was necessary to use fertilized females, and the following procedure was adopted. A number of recently emerged flies were placed in a cage and fed on meat, sugar and water until oviposition began; the meat was then taken away and at intervals females were removed and placed separately in test-tubes containing meat. Those that oviposited were then transferred to incubators; each fly was kept in a separate jar and the times of oviposition were noted.

Table I

Effect of temperature on the initial development of the ovaries in recently emerged females

Temperature (°C.)	Days required for development of mature eggs
37	2
30	3
23	4·5
19·5	7
15–16	13

Table II

Effect of temperature on the production of eggs by fertilized females

	Interval in days between 1st and subsequent ovipositions	
Temperature (°C.)	Limits	Average value
37	1·0–1·5	1·2
30	1·0–1·7	1·4
23	2·0–3·0	2·4
19·5	2·9–3·8	3·3
15–16	4·5–8·0	5·5

Table I shows the effect of temperature on the development of the ovaries in recently emerged females. These values are in good agreement with those obtained by Mackerras (1933) and Evans (1935) with this species. Mackerras found mature eggs after 5–6 days at 20–22° C., Evans after 6 days at 23° C. Table II shows the results obtained with fertilized

[1] In an experiment with flies fed on dung in jars, one of the flies died from an infection with *Empusa* species. At about the same time (November 1937) all the flies in one of the cages died from the same cause.

females which had oviposited once. It will be seen that, at corresponding temperatures, the second and subsequent batches of eggs develop in about half the time required by the first batch. With regard to the extreme temperatures used, there was evidence that these were unfavourable. At 37° C. mortality was high, and at 15–16° C. oviposition was irregular, the interval increasing considerably after the third oviposition. The figures shown in Table II for this temperature refer only to the first three ovipositions. At other temperatures, the intervals were regular except that they tended to decrease slightly after the second oviposition. It should be noted that these figures represent minimum values since the flies were kept under optimum conditions of nutrition. In nature, the development of the ovaries will be slower as the fly has to search for food.

Effect of nutrition on the development of the ovaries

Although previous workers have shown that a meat diet is essential if the eggs are to develop in the ovaries, no critical examination appears to have been made on the minimum number of meat meals necessary. In order to investigate this point, some recently emerged (unfed) females were divided into three groups and kept in jam jars at 23° C., sugar and water forming the basal diet. Meat was fed by placing the flies separately for 4 hr. in test tubes containing liver; generally, the fly had finished feeding after 2–3 hr. The first group received one feed of liver on the second day, the second group two feeds on the second and fifth days, the third group three feeds on the second, fourth and sixth days. On the eighth day all the flies were killed and dissected. It was found that the ovaries were only partly developed in the flies receiving one meal of liver; mature eggs were present in flies which had been given two or three meals. When beef muscle was substituted for liver, mature eggs were found in flies receiving three meals but only in some of the flies receiving two meals. This difference was probably due to the fact that flies cannot extract so much juice from muscle (butcher's meat) as from liver; this was shown to be so by weighing flies before and after feeding on muscle and liver. Clearly, at least *two* meat meals are necessary for the development of mature eggs in recently emerged flies. Similar experiments were carried out with fertilized females that had oviposited once. These showed that two meat meals are necessary, also, for the regrowth of eggs after oviposition; this includes the meal taken when laying; i.e. if a gravid female was allowed to feed and oviposit on liver, mature eggs were not found later unless at least one meat meal was given in the interim.

In these experiments the flies were always kept for at least 6 days at 23° C. after the first meat meal, this period being adequate for egg development on a meat diet (Tables I and II). Yet in all cases where immature eggs were found owing to insufficient meat, the eggs had reached the same stage of development. Under normal conditions, the eggs first appear in the ovaries as small spheres; later they become oval, then elongate further and become opaque. In flies which had received only one meat meal, the eggs were always found to be in the small spherical stage and never partly elongated. This indicates that the process occurs in two stages, and that the second stage, which includes elongation, does not begin until the fly has obtained sufficient meat for the eggs to become mature.

In order to find out how much juice a fly takes when feeding on liver, females were starved overnight and then weighed before and after feeding. It was found that a fly may consume as much as 15 mg. of juice. Since two meals are required for the development of a batch of eggs, not more than 30 mg. of liver juice are necessary in addition to carbohydrate.

Feeding experiments were also carried out to determine whether meat can be replaced by other substances. These tests were carried out in jam jars, as before, the flies being fed for a week on sugar, water and the test substance, and then dissected and examined. Experiments were made both with recently emerged flies and ones which had already oviposited. In some cases tests were carried out in a similar way in cages. The results are shown in Table III.

Table III

The effect of different foods on the development of the ovaries

Substance added to basal diet of sugar and water	Development of ovaries
Meat	+
Blood serum	−
Blood serum + marmite	+
Milk	−
Sheep dung	−
Dog dung	−
Pollen	−
Ivy flowers	−
Blackberries	−

These findings are in agreement with those of previous workers; thus, Mackerras (1933) found no development of the ovaries in blow-flies fed on various plant products or sheep dung. In the present experiments faeces from a carnivorous animal were included, but these also gave

negative results. It will be seen that none of the plant materials tested can replace carrion. The pollen used was obtained from pollen cells in beehives. Ivy flowers were tested as it was noticed that these attract blow-flies in considerable numbers. The results with serum suggest that the food requirements of the female blow-fly include salts and vitamin B in addition to protein. Blood serum is rich in protein of good quality, but deficient in potash, phosphate and vitamin B (Hobson, 1935*a*). Marmite supplies these substances, and Table III shows that eggs develop in flies fed on serum if this is supplemented with marmite. Mackerras (1933) has shown that a *L. sericata* female may produce as many as 2000 eggs, i.e. at least four times its own weight. Clearly, all the necessary constituents must come from the food. When carbohydrate is supplied in the form of pure cane sugar, as in these experiments, the meat must supply salts and vitamins in addition to protein. Under natural conditions this may not be so, since the fly obtains its sugar from plant juices which contain also water-soluble vitamins and salts. The failure of flies to produce eggs when fed on milk is interesting since this is usually regarded as a complete foodstuff; this result suggests that blow-flies may require specific accessory factors present in meat.

Chemotropic responses of gravid females

This subject is of great fundamental importance since the attraction of sheep for *L. sericata* is the initial cause of maggot infestation. Observations in the field have shown that the attraction is twofold; one factor is supplied by the sheep and the other by putrefying material or certain products of putrefaction, such as ammonium carbonate, indole or skatole (Hobson, 1935*b*; 1936). Furthermore, this attraction is specific for gravid females of *L. sericata*; it is therefore necessary to use gravid females for studying this response in the laboratory. The method used for obtaining gravid females was essentially the same as that described by Evans (1935). Recently emerged flies were fed on sugar and water for 2–3 days; meat was then supplied until the first oviposition occurred, when the meat was removed and the flies fed only with sugar and water. Examination showed that most of the females contained ripe eggs after this treatment. However, the readiness of flies to oviposit varied greatly in different cultures. It was always increased by leaving the flies for several days after removing the meat; also flies oviposited more readily in the summer, probably because they feed better in the presence of sunlight. As a rule, the experiments on oviposition were carried out during the second week after the end of meat feeding.

If gravid females are kept without meat, they occasionally lay on the sides of the cage, on wet cotton-wool, or on sugar, particularly if this has started to ferment. Various substances were, therefore, exposed in the cages to attract oviposition. The earlier experiments gave negative results, but later, with cultures bred during the summer, certain putrefactive products were found to attract females strongly and to stimulate oviposition. This response, however, was very uncertain, and it was found that oviposition could be induced far more readily by placing females in tubes containing the test substances. Liquids were absorbed on cotton wool, which was also used to plug the tubes; the flies were placed in an incubator at 23° C. for several hours, the tubes then being examined for the presence of eggs. Table IV gives a list of substances which stimulated oviposition.

Table IV

List of substances found to stimulate oviposition by L. sericata

Indole	Ammonium carbonate
Skatole	Ethyl alcohol
Ammonia	Suint
Trimethylamine	

In addition, sheep's wool moistened with water occasionally provoked oviposition; however, in control tests with damp cotton wool oviposition also occurred with some cultures. The proportion of flies found to oviposit on these substances was small, about 1 in 10; with the substances tested in Table IV usually four to eight out of ten flies oviposited. The sample of suint used was a water extract of wool which had stood for several months in the laboratory and had decomposed. Oviposition was sometimes found with suint, indole and trimethylamine solutions when exposed in cages, but none of these substances attracted oviposition when exposed in the field. It was found that gravid females readily oviposited on sheep. If the flies were released near sheep, they immediately flew away; but if their wings were cut and they were placed in the fleece, near a pad of cotton-wool soaked in indole solution, they usually oviposited within half an hour. Oviposition experiments were also carried out with wild flies (*L. sericata*) trapped by means of sheep treated with a solution of indole. These flies oviposited on indole or suint, and responded more readily than bred flies. In fact difficulty was experienced in transporting the flies to the laboratory as many oviposited in the tubes before they had been tested.

These observations show that the oviposition response consists of two distinct phases: (1) attraction from a distance, and (2) stimulation to oviposit. The first depends upon two factors in the case of sheep, supplied by the live animal and by products of protein decomposition (Hobson, 1935*b*, 1936). Similarly, the attraction of carrion may consist of putrefactive products combined with other odoriferous substances present in carrion. The second phase, oviposition, depends upon several factors; although it can be produced by certain chemicals, it is also a tactile response. A meal often seems to stimulate oviposition, probably because it distends the abdomen and the ovipositor is then more liable to touch the object. For this reason it is usually easier to provoke oviposition on meat than on other materials which do not supply food.

Effect of nutrition on oviposition

Experiments on oviposition in *L. sericata* are rendered difficult by the variation in response even among flies bred in the same cage under identical conditions. When the flies were kept in the dark in jars, as in the temperature experiments, oviposition occurred at regular intervals. Light, therefore, has no direct effect, but under these confined conditions, being very close to the food, the flies were always gorged with food. When kept in cages, flies are more active and feed more freely at high temperatures and in bright light. Differences in nutrition were probably the cause of the variation in oviposition responses and the better results obtained in summer. Direct evidence for this was obtained from experiments with fertilized flies, which, after laying on liver, were kept in jars and fed with liver every second day. Oviposition occurred at the third meal in some cases, i.e. when two meals had been digested since the previous oviposition. However, eggs were only laid in response to indole by flies that had received three meat meals since the last oviposition. This suggests that a diet rich in meat is necessary before a female will lay on substances other than carrion. This was shown also by feeding gravid females on meat for a short time and testing them the next day with indole; the meat-fed flies laid more readily than females from the same cage which had not received meat.

DISCUSSION

The results of the nutrition experiments with blow-flies indicate that carrion plays an important part in the infestation of sheep with maggots. Carrion seems to be an essential food for the development of the ovaries in the female, since it cannot be replaced either by animal excreta or

plant materials. Theoretically, then, fly attack might be controlled by the destruction of all carrion, but the following considerations will show that this means of control is impracticable, apart from the question of cost. *L. sericata* has a flying range of at least 4 miles according to Gurney & Woodhill (1926), and a very small animal, such as a dead bird or mouse, would supply innumerable flies with sufficient food for the development of the ovaries. It is of interest, in this connexion, to calculate the amount of food required by the female fly for the development of ripe eggs and by the larvae emerging from these eggs. The fly requires two meals, i.e. about 30 mg. of meat juice. Salt (1932) has shown that each larva requires at least 150 mg. of meat for full development. If the fly lays 100 eggs, the emerging larvae will require 15,000 mg., apart from competition from larvae of other blow-flies. It is clear that a scarcity of carrion will react far more on the larval stage than on the fecundity of the adults. However, carrion is probably not a limiting factor in the case of *L. sericata* larvae since live sheep are an alternative breeding place; larvae growing on sheep do not suffer from competition with other species or from the attack of parasites (Davies, 1930).

Although, owing to the abundance of small dead animals, complete destruction of carrion is impracticable, attention should be paid to the more important sources of carrion, so as to prevent the population of gravid blow-flies becoming excessive. These sources include dead farm animals, rabbit warrens, meat exposed in shops or in carts during transit. Macleod (1937) has recently suggested trapping by the old-fashioned method of hanging carrion over water, into which the maggots eventually drop and are drowned. Although this method traps the eggs, it may do more harm than good since it supplies the adults with food without catching them.

With regard to the oviposition experiments, an interesting point is the difficulty of rearing gravid females which are ready to oviposit. A diet rich in meat is essential if flies are to oviposit on some object other than carrion, but meat must not be kept too long in the cages, otherwise the females oviposit and are no longer gravid. Yet in the field during the summer gravid females are quickly attracted in large numbers to a sheep infested with maggots or treated with indole. Before a fly can oviposit on a sheep, it must have at least two, perhaps three, meals of carrion. Why, if it can find carrion for feeding purposes, does the fly not lay its eggs on carrion? There seems to be three possible explanations: (1) Carrion is a limiting factor, and blow-flies have difficulty in finding carrion for feeding and oviposition. Females (*L. sericata*) which become gravid

are, therefore, liable to be attracted to sheep owing to lack of carrion. (2) Carrion is abundant and the females are continually becoming gravid, ovipositing and becoming gravid again. Owing to the high population of *L. sericata* and their keen scent, gravid females soon find their way to a sheep that has become attractive. If a gravid female is not quickly attracted to sheep, it may oviposit on carrion, but it soon becomes gravid again owing to abundance of food. (3) *L. sericata* is attracted to carrion only for feeding purposes and not to oviposit.

The first explanation seems unlikely since the amount of carrion required by the adult for feeding and as a focus for oviposition, is small compared with that needed by the larva. Only an abundance of carrion, leading to the production of large numbers of flies, followed later by a dearth, would make carrion a limiting factor of adult fecundity. The second and third explanations are, therefore, more likely; also, they are not incompatible. The relative attractiveness of sheep and carrion for gravid females is clearly an important factor. This might be tested by examining the proportion of gravid and non-gravid females visiting carrion, comparison being made between *L. sericata* and other species which breed only on carrion; or by determining what proportion *L. sericata* forms of the total catch in meat traps of (1) eggs, (2) adults. It seems possible that females of this species may frequent districts rich in carrion when they are not gravid, migrating to sheep grazings when gravid. Thus, it has been shown that the attraction supplied by sheep is specific for gravid females (Hobson, 1936). Also, Morison (1937) found that *L. sericata* formed only 3% of the blow-flies caught in meat traps and he obtained evidence that this species is more abundant in the proximity of sheep.

Although *L. sericata* readily oviposit on carrion under cage conditions, it does not necessarily follow that carrion is highly attractive to gravid females in the field. The present experiments have shown that the oviposition response normally consists of two distinct phases, attraction from a distance and stimulation to oviposit. Indole, for example, stimulates gravid females to oviposit, but does not attract them (except in conjunction with live sheep). The oviposition experiments have shown that various putrefactive products stimulate oviposition; little progress, however, has been made in finding substances which will attract gravid females (without live sheep) in the field.

SUMMARY

1. An investigation has been made of the effect of temperature upon the time required for the development of the ovaries in the sheep maggot fly.

2. It has been shown that two meat meals are essential for the development of mature eggs in the ovaries. This applies also to the further production of eggs after oviposition.

3. Neither plant materials nor animal excreta can replace meat in the diet of the female fly if eggs are to develop in the ovaries.

4. Various products of putrefaction stimulate gravid females to oviposit, but these substances do not attract flies from a distance.

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SOME APPLICATIONS OF LABORATORY BIOLOGICAL TESTS TO THE EVALUATION OF FUNGICIDES

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CONTENTS

INTRODUCTORY

IN a recent paper (Horsfall *et al.* 1937) the factors determining the field performance of a fungicide are separated into two groups. Included in the first group are the quantity factors governed by physical and chemical properties which determine the retention and tenacity of the spray deposit and, thus, fix the amount and evenness of distribution of the material on the substratum. The second group constitutes the quality factors making up fungicidal value. Fungicidal value is defined as the resultant of (1) the ease of formation of an active fungicide from the spray deposit (i.e. availability), and (2) the relative inherent toxicity of the active material thus produced. Recent work by McCallan & Wilcoxon (1936) has suggested a relation of availability to the presence

of solubilizing spore excretions. For the present, however, fungicidal value cannot be assessed on purely chemical data, and a biological technique is required for the double purpose of rendering the fungicide available and registering the toxicity of the compound produced.

The use of fungus spores in such biological tests is a generally accepted procedure which has the advantage of rapidly giving results that can be simply expressed and readily compared. When the aim is the evaluation of materials as protectant fungicides, the approved method is that of sowing spores in contact with the dried residue of a fungicide applied by a standardized spraying technique (McCallan, 1930).

In the present investigation, this method has been used for a sorting out, in terms of relative protectant value, of a number of sulphur and copper compounds, for many of which fungicidal properties had been claimed. Further, this technique has been employed for giving a biological demonstration, paralleling the chemical proof, of the *in vitro* effect of certain spray supplements on the tenacity of fungicidal spray deposits.

In considering, however, the absolute concentration of a fungicide (with or without supplement) necessary to give control of a specific disease in the field, the applicability of results from *in vitro* tests remains in question (Montgomery & Moore, 1938). A laboratory method of testing fungicides on living leaves has been suggested in a previous paper (Marsh, 1936), and the results of a series of such tests are given below. A limited number of experiments have also been made to determine the amount of agreement between the results of the laboratory leaf tests and those of field trials using the same organisms and materials.

Methods

In carrying out the laboratory tests the materials for examination, after dilution to the required strength, are kept agitated by a mechanical stirrer and sprayed on to cellulosed slides by means of an atomizer operating under standard conditions. The apparatus is fully described by Evans & Martin (1935) and its employment in fungicide tests by Marsh (1936). Spraying is commonly carried out for either 5 or 10 sec. at a pressure of 2 atm., the cellulosed slide being held 2 ft. from the jet. The deposition for a 10 sec. exposure is at the rate of 0·008 ml. spray per square centimetre.

The sprayed slides are dried at laboratory temperature and maintained exposed to the air but protected from dust for at least 24 hr. If

the tenacity of the spray deposit is to be tested, the slides are then held 10 in. from the atomizer jet for 60 sec., and subjected to a distilled water spray at 1½ atm. pressure. After this leaching process the slides are again allowed to dry before being used for a spore germination test.

In carrying out a test the slides are placed on glass racks in moist chambers (McCallan, 1930). On to each slide is then pipetted three separate drops of spores suspended in water. Each drop is approximately 0·04 ml. and contains 150–200 spores; its area of spread on the sprayed slide is a circle of about 7 mm. diameter. In this investigation the spores most frequently employed have been conidia of the Pear Scab fungus (*Venturia pirina*) obtained from young naturally-occurring leaf infections on the variety Williams' Bon Chrétien. Other spores used have been conidia of *V. inaequalis* (from leaves of Crimson Cox apple) and ascospores of *Nectria galligena* (from natural infections on Lane's Prince Albert wood). The sown slides were incubated at 21° C. and the percentage of germinated spores was determined by counting after 48 hr. Each determination was made on at least 600 spores distributed in six drops on a pair of slides.

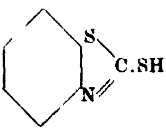
Tests of certain of the materials were also made on individual leaves in the laboratory using the technique described elsewhere (Marsh, 1936). Young leaves of Crimson Cox apple were used for the tests with *Venturia inaequalis* and those of Williams' pear for tests with *V. pirina* spores.

MATERIALS

(i) *Rubber accelerators and allied compounds*

In vulcanizing rubber by the addition of sulphur it is the practice to expedite the reaction by the employment of certain thiazole, thiuram and guanidine derivatives which are referred to under the term of rubber accelerators. These complex organic compounds are accordingly available in commerce and, for certain of them, fungicidal and/or insecticidal properties have been claimed. Further, they are of interest in connexion with the mechanism of the fungicidal action of sulphur. The materials tested are listed below with notes of any previous references to their fungicidal and insecticidal properties.

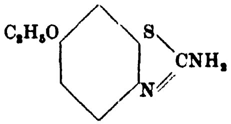
(1) *Mercaptobenzthiazole*:



Patent specifications claim that this material is a disinfectant for seeds, corms and tubers, and that a 0·1% solution of the sodium salt

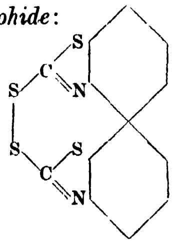
inhibits the growth of cultures of *Botrytis cinerea*, *Phoma pomi*, *Glomerella cingulata*, *Sclerotinia cinerea* and *Fomes annosus*. Insecticidal properties are also claimed (see Roark & Busbey, 1935). Montgomery & Moore (1938) found that mercaptobenzthiazole was fungicidal to *Venturia inaequalis* spores at 0·01 %.

(2) *6-ethoxy-2-aminobenzthiazole*:



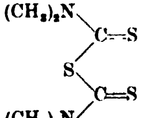
The fungicidal and insecticidal claims made for a 0·1 % solution of the hydrochloride of this compound are similar to those made for mercaptobenzthiazole.

(3) *Dibenzthiazyl disulphide*:

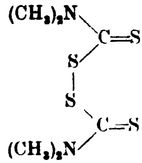


No records of previous fungicidal tests with this material have been found.

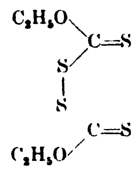
(4) *Tetramethyl thiuram monosulphide*:



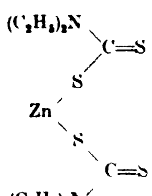
This material has been patented as a seed disinfectant and as a timber and textile preservative to be used at 0·2 %. It is also specified as an insecticide (see Roark & Busbey, 1935). Tests made at Newark, Delaware (Guy, 1937), show that it acts as a repellent to leaf-eating insects. In field tests against Apple Scab at East Malling (Montgomery *et al.* 1935) it was used at 0·07 %, at which concentration it was inferior in fungicidal power to lime sulphur at standard strength. In laboratory tests (Montgomery & Moore, 1938) against Apple Scab spores it was found to be fungicidal at 0·005 %.

(5) *Tetramethyl thiuram disulphide*: 

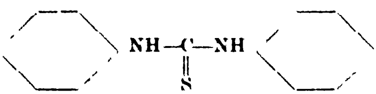
A saturated aqueous solution of this material is said to act as a wood preservative. Added to an agar medium at the rate of 1 part in 250 it inhibited the growth of *Aspergillus niger* and *Fomes annosus* (see Roark & Busbey, 1935). In a field trial at East Malling it was shown to be non-phytocidal on apples at 0·25% (Moore *et al.* 1936). At Newark, Delaware, it is recorded as a repellent (Guy, 1937). Montgomery & Moore (1938) found that it was fungicidal to *Venturia inaequalis* spores at 0·0005%.

(6) *Xanthic disulphide* (synonym—Ethyl dixanthogen): 

This material is not in use as a rubber accelerator but was included in the tests as a compound closely related in structure to the foregoing. Montgomery & Moore (1938) found that the methyl homologue was not fungicidal to *Venturia inaequalis* spores at 0·1%.

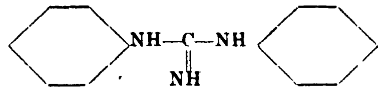
(7) *Zinc diethyl dithiocarbamate*: 

No record of fungicidal tests with this material has been found.

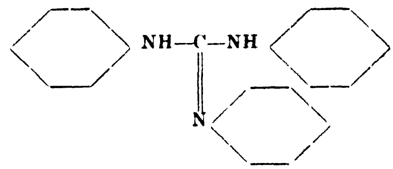
(8) *Thiocarbanilide*: 

This material is stated to be poisonous to leaf-eating insects; it has been suggested for use as a dust on fruit trees in the proportion of 15 parts of thiocarbanilide to 85 parts of sulphur. It is also claimed that a dust containing 3–10% thiocarbanilide may be used on wheat plants to control *Puccinia graminis* (see Roark & Busbey, 1935).

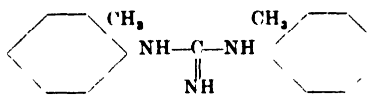
(9) *Diphenyl guanidine*:



(10) *Triphenyl guanidine*:



(11) *Diorthotolyl guanidine*:



No reference has been found to the fungicidal use of these materials. The related compounds, benzthiazole guanidine and benzoxazole guanidine were tested by Wilcoxon & McCallan (1935) who record that they are not promising fungicides.

Materials 1–11 listed above were supplied by Messrs Imperial Chemical Industries, Ltd., in the form of finely divided powders. As these materials are insoluble or very slightly soluble in water they were diluted for testing by dissolving in a minimum quantity of acetone and adding the solution to water containing methyl cellulose as a dispersing agent. The solvent is removed by evaporation on the drying of the spray deposit.

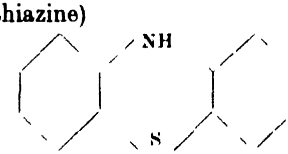
(ii) *Thiocyanates and thiodiphenylamine*

(12) *Lauryl thiocyanate*: $C_{12}H_{25}.SCN$.

This material is in commercial use as an ovicide and acaricide. No reference to fungicidal tests has been found.

(13) *Cetyl thiocyanate*: $C_{16}H_{33}.SCN$.

(14) *Thiodiphenylamine*: (synonym—Phenothiazine)



This material is of importance in dyestuffs manufacture as the parent substance of a series of dyes including methylene blue. As an insecticide it is highly specific but is considered one of the most important synthetic organic compounds for possible use as a substitute for arsenicals. Tested

in dust form as a fungicide it was found non-toxic to spores of *Sclerotinia fructicola*, *Botrytis paeoniae* and *Pestalotia stellata* (Wilcoxon & McCallan, 1935). Against spores of *Venturia inaequalis* it is recorded as effective at 0·1% (Montgomery & Moore, 1938).

The thiocyanates were received in a petroleum oil emulsion. This mixture was not found satisfactory for testing as the emulsion itself showed toxic properties. Further tests were made using the thiocyanates in a sulphite lye emulsion.

Commercial thiodiphenylamine was obtained from three different sources, and a sample of pure steam-distilled thiodiphenylamine was also employed. These materials were diluted for testing either by solution in acetone as previously described, or by grinding up the solid with sulphite lye syrup, and then diluting with water to form a suspension.

(iii) *Copper compounds*

(15) *Cuprous cyanide.*

This material has been successfully employed as an insect repellent but has not found commercial application because of potential hazards to human health. Records of fungicidal tests against spores of *Venturia inaequalis* are given in a previous paper (Marsh, 1936). Phytocidal effects on apple are recorded by Montgomery *et al.* (1935) and by Marsh *et al.* (1937).

(16) *Cupric ammonium silicate.*

Experiments in New Jersey have shown that a range of copper ammonium silicates can be prepared having differing copper availabilities. From this range a material was selected by Sessions (1936) which gave excellent control of apple scab in a field trial.

(17) *Cuprous thiocyanate.*

(18) *Cupric oxalate.*

(19) *Cupric phthalocyanine.*

The last named has been employed as a pigment: no insecticidal or fungicidal properties have been claimed for this or for the two preceding compounds.

In the present investigation, Nos. 15–19 were obtained as paste suspensions from Messrs Imperial Chemical Industries, Ltd. For testing they were diluted with water to give concentrations of 0·1% copper and 0·2% copper.

(20) *Cuprous iodide.*

This material was obtained from Drs Fajans and Martin as a fine suspension in water (see Fajans & Martin, 1937, for method of preparation).

(21) *Cuprous oxide.*

The fungicidal value of cuprous oxide is fully discussed elsewhere (Horsfall *et al.* 1937). The material used for tests was a suspension of cuprous oxide in cotton-seed oil emulsified with sulphite lye. The concentrate contained 20·7% copper and 41% cotton-seed oil. It was diluted for use to a strength equivalent to 0·1% copper.

(iv) *Spray supplements*

The following materials were employed:

Sulphonated lorol	Gelatine
Agral 2	Lime casein
Methyl cellulose	Petroleum oil emulsion
Sulphite lye	Cotton-seed oil

Descriptions and specifications of these materials have already been given by Martin and his co-workers (Evans & Martin, 1935; Fajans & Martin, 1937).

Results

The results obtained fall into two groups:

(1) Sorting-out tests of individual materials.

(2) Tests relating to the effects of the incorporation of spray adjuvants.

Concerning the second group, certain data are available relating the results of the tests to chemical findings on the one hand and to conclusions from field trials on the other.

(i) *Sorting-out tests*

The results of tests using the rubber accelerators and other sulphur-containing compounds are given in Table I, the test spores employed being the conidia of *Venturia pirina* except where otherwise stated. In this table, a concentration of a material which, after being sprayed on a slide and allowed to dry, completely inhibited germination in the drops of spore suspension sown upon it, is termed fungicidal, represented by the symbol *F*. If the mean percentage germination in the test drops lay between 0 and 20, the concentration tested is considered just below

fungicidal strength (represented by *f*). Germinations between 20 and 60% indicate a weakly fungicidal concentration (*wf*) and above 60% no fungicidal value (*N*).

Table I

Tests with rubber accelerators and other sulphur-containing materials

All tests made with slides sprayed for 5 sec.

Material	Fungicidal value at 0·1%	0·05%	0·01%
Diphenylguanidine	—	*F*	*f*
Diorthotolylguanidine	—	—	*f*
Tetramethylthiuram disulphide	—	*F*	*wf*
Tetramethylthiuram monosulphide	—	—	*wf*
Thiocarbanilide	—	*f*	*wf*
Thiodiphenylamine	*F*	—	*wf*
Mercaptobenzthiazole	*F*	*wf*	*N*
Zinc diethyldithiocarbamate	*F*	—	*N*
Xanthic disulphide	—	—	*N*
6-ethoxy-2-aminobenzthiazole	—	—	*N*
Triphenylguanidine	—	—	*N*
Dibenzthiazyl disulphide	—	*N*	—
Cetyl thiocyanate	—	*N*	—
Lauryl thiocyanate*	—	*N*	—

* Tested against conidia of *Venturia inaequalis*.

The first six materials given in Table I retain a measure of toxicity even when applied at 0·01%. Of these materials the two guanidine derivatives, diphenylguanidine and diorthotolylguanidine, are slightly soluble, and thiocarbanilide is also very slightly soluble. This factor would enhance the availability of these compounds but at the same time detract from their usefulness as protectants. The remaining materials showing promise are the thiuram sulphides and thiodiphenylamine. As previously recorded, tetramethylthiuram monosulphide has been tested in the field at East Malling and showed little promise as a fungicide.

Thiodiphenylamine and diphenylguanidine were selected for submission to laboratory leaf tests using *Venturia inaequalis* as the test organism. Leaves of Crimson Cox apple were sprayed with a 0·1% suspension of each compound in 0·2% sulphite lye. The spore germinations recorded in ten drops, on six leaves sprayed with thiodiphenylamine were as follows: 72, 84, 82, 70, 82, 69, 80, 72, 78 and 74%. The figures for four drops on the four leaves, sprayed with diphenylguanidine were: 82, 70, 84, and 84%. It was concluded that neither material, at 0·1% concentration, was of sufficient fungicidal value on leaves to be given a field trial.

The other material of special interest is lauryl thiocyanate which was shown by the slide tests to be of no fungicidal value.

Table II gives the results of tests with certain copper derivatives. In a previous paper (Marsh *et al.* 1937) the distribution of fungicidal properties among a wide range of copper compounds is noted, the principal criterion used being the control of Potato Blight in field trials. Extensive field and laboratory trials have also been made with the copper oxides (Horsfall *et al.* 1937) and certain results of toxicity trials against apple scab spores with the cyanide and ferrocyanide have been recently published (Marsh, 1936). The last-named figures were extracted from a series which is given complete in Table II.

Table II

Tests with copper compounds on slides

Material	Spray exposure sec.	Fungus spores	Fungicidal value at 0·2% Cu	0·1% Cu
Cuprous cyanide	3	*V. pirina*	*F*	*f*
,,	10	,,	—	*f*
,,	10	*V. inaequalis*	*F*	-
Cupric oxalate	10	,,	*F*	
Cupric ferrocyanide	10	,,	*F*	
Cuprous sulphite	10	,,	*F*	
Cupric ammonium silicate	10	,,	*f*	-
Cuprous thiocyanate	10	,,	*N*	
Copper phthalocyanine	10	,,	*N*	

The first four compounds in Table II were further tested on leaves in comparison with Bordeaux mixture with the following results:

Table III

Tests with copper fungicides on leaves

All leaves sprayed for 10 sec.; conidia of *V. inaequalis* used as test spores.

Material	Fungicidal value at 0·2% Cu
Bordeaux mixture	*F*
Cupric oxalate	*f*
Cuprous sulphite	*vf*
Cuprous cyanide	*vf*
Cupric ferrocyanide	*N*

Because of its combined insecticidal and fungicidal value, cuprous cyanide was selected for a test on the field scale. The results are given in Table XI.

(ii) *Tests using spray supplements*

This section includes the results of laboratory biological tests made to examine the effects of supplements on the tenacity of spray deposits. Such effects have been determined chemically by Fajans & Martin (1937)

using cuprous iodide and cuprous oxide sprays, and comparisons with the chemical findings are given in § (iii). The majority of the laboratory biological tests have been with materials also employed in field trials and, where comparisons with the latter are possible, they are given in § (iv).

(1) *Water-soluble spray supplements.*

(*a*) *Agral* 2. Tables IV A and IV B summarize the results obtained with the use of fungicides together with Agral 2. Where the number of replications is small all the percentage germinations are given; otherwise the figures are expressed as the mean percentage and the standard error of the mean.

Table IV A

Results on slides using fungicides with Agral 2

Fungicide	Concentration of Agral 2	Fungus spores	% germination on slides: Unleached	Leached
Bordeaux mixture (0·025% Cu)	None	*N. galligena*	0, 0	0, 0
			0, 0	0, 17
			0, 0	0, 0
,,	0·05%	,,	0, 1	10, 0
			0, 0	0, 0
			0, 0	6, 16
Lime sulphur 1%	None	*V. inaequalis*	0, 0	0, 0
			0, 0	0, 0
			0	0
,,	0·05%	,,	0, 0	0, 0

Table IV B

Results on leaves of Crimson Cox apple using Agral 2 as spray supplement

Fungicide	Concentration of Agral 2	Fungus spores	No. of results	Mean % germination on leaves: Unleached	Leached
Lime sulphur 1%	None	*V. inaequalis*	35	21 ± 4·8	—
,,	None	,,	41	—	13 ± 2·8
,,	0·05%	,,	22	20 ± 2·7	—
,,	0·05%	,,	34	—	22 ± 4·5

The difference between the pair of means in the final column is 9 ± 5·3, i.e. the difference is not significant. The conclusion is that the tenacity of the lime sulphur deposit on the leaves is not reduced by the presence of the Agral 2 to any extent that is recorded by the spore germination figures.

(*b*) *Sulphite lye.* Table V gives the results obtained from tests made on slides sprayed with various fungicides, with and without sulphite lye.

Table V

Results on slides using sulphite lye as supplement

Fungicide	Concentration of sulphite lye (as syrup)*	Fungus spores	% germination on slides	
			Unleached	Leached
Lime sulphur 1%	None	*V. inaequalis*	0	0
,,	0·75%	,,	0	16
,,	None	*V. pirina*	0	0
,,	0·75%	,,	0	68
Cuprous iodide 0·01%	None	*V. inaequalis*	6, 0	3, 0
,,	0·5%	,,	0	79
Cuprous oxide 0·1% Cu	0·15%	*V. pirina*	0, 5, 2	85, 86, 86
Bordeaux mixture 0·025% Cu	None	*N. galligena*	0, 0	17, 17
			0, 0	0, 0
,,	0·5%	,,	6, 3	71, 49
			0, 0	41, 33

* Sp.gr. 1·30, containing approx. 50% solid sulphite lye.

The deleterious effect on tenacity of 0·5–0·75% sulphite lye is reflected by the high percentage germination recorded for the leached slides on which this supplement was used. No detailed laboratory trial on leaves has been made using sulphite lye alone as a supplement, but results are given in Table VII B of leaf tests made with an oil emulsion supplement in which sulphite lye was the emulsifier.

(*c*) *Other water-soluble supplements.* Sulphonated lorol, methyl cellulose, lime casein and gelatine were tested on slides as supplements for the cuprous iodide suspension prepared by Fajans & Martin by the method described elsewhere (Fajans & Martin, 1937). The results of a comparative test are given in Table VI.

Table VI

Results on slides using various water-soluble supplements

Fungicide	Supplement	Fungus spores	% germination on slides	
			Unleached	Leached
Cuprous iodide 0·01%	None	*V. inaequalis*	6, 0	3, 0
,,	Gelatine 0·05%	,,	0	0
,,	Lime casein 0·05%	,,	0	0
,,	Methyl cellulose 0·05%	,,	0	20
,,	Sulphonated lorol 0·05%	,,	0	0

Under the conditions of this experiment, the use of methyl cellulose was attended by a slight loss in tenacity: the other supplements showed no adverse effect.

(2) *Oils and oil emulsions used as supplements.*

(*a*) *White oil-sulphite lye emulsion.* The emulsion employed in these tests contained 66% by volume of highly refined (Grade G) petroleum

oil, 7 % sulphite lye syrup and 27 % water. The method of compounding the emulsion is described by Kearns & Martin (1936). The results of the tests made on slides and on leaves, respectively, are given in Tables VII A and VII B.

Table VII A

White oil emulsion as supplement: results on slides

Fungicide	Concentration of oil emulsion	Fungus spores	% germination on slides: Unleached	% germination on slides: Leached
Bordeaux mixture 0·025 % Cu	None	*N. galligena*	0, 0, 0	0, 0, 0
,,	7½ %	,,	2, 3, 2	12, 73, 64

Table VII B

White oil emulsion as supplement: results on leaves

Fungicide	Concentration of oil emulsion	Fungus spores	No. of results	% germination on slides: Unleached	% germination on slides: Leached
Lime sulphur 1 %	None	*V. inaequalis*	35	21 ± 4·8	—
,,	None	,,	41	—	13 ± 2·8
,,	1 %	,,	26	14 ± 2·8	—
,,	1 %	,,	43	—	40 ± 4·7

There is a significant difference between the means in the final column—27 ± 5·5—indicating that, under the conditions of these tests, the addition of the emulsion somewhat reduced the tenacity of the lime sulphur. It should be noted that the diluted spray contained sulphite lye at a concentration of approximately 0·07 %.

(*b*) *Cotton-seed oil*. Two methods of using cotton-seed oil as a supplement have been employed in these tests. With Bordeaux mixture (Tables VIII A, VIII B) the oil as such has been added to the diluted fungicide and the mixture strongly agitated, when the Bordeaux precipitate itself acts as the emulsifier. In the tests with cuprous oxide

Table VIII A

Cotton-seed oil as supplement: results on slides

Fungicide	Concentration of oil	Fungus spores	% germination on slides: Unleached	% germination on slides: Leached
Bordeaux mixture 0·025 % Cu	5 %	*N. galligena*	0, 6, 30	0, 7, 14
Bordeaux mixture 0·1 % Cu	None	*V. pirina*	8, 4, 2	2, 0, 1
			0, 0, 0	0, 0, 2
,,	0·25 %	,,	0, 0, 0	0, 7, 6
			7, 10, 11	12, 36, 26
			0, 0, 0	1, 0, 0
			0, 0, 0	0, 0, 0

Table VIII B

Cotton-seed oil as supplement: results on leaves of Williams' pear

Fungicide	Concentration of oil	Fungus spores	No. of results	Mean % germination on leaves: Unleached	Leached
Bordeaux mixture 0·1 % Cu	0·25 %	*V. pirina*	32	2 ± 0·86	—
,,	0·25 %	,,	32	—	2 ± 0·73

(Table IX), the oil was first emulsified with sulphite lye and then compounded with the oxide to give a paste containing 23 % cuprous oxide and 41 % cotton-seed oil. This was then diluted for use as required.

The results given in Table VIII A show that the addition of cotton-seed oil in these experiments had no adverse effect on tenacity. Table IX gives the figures obtained on leaves, using the cuprous oxide—cotton-seed oil emulsion at a copper concentration of 0·1 % and an oil concentration of 0·25 %.

Table IX

Cotton-seed oil emulsion as supplement: results on leaves of Williams' pear

Leaf	Spray	Exposure sec.	Fungus spores	% germination on leaves: Unleached	Leached
A	Cuprous oxide	5	*V. pirina*	15, 28	—
B	in cotton-seed	5	,,	18, 16	—
C	oil emulsion	5	,,	—	40, 54
D		5	,,	—	61, 24
E		2½	,,	66,	—
F		2½	,,	64, 68	—
G		2½	,,	—	86, 68
H		2½	,,	—	92, 87

Table IX demonstrates some loss in tenacity upon leaching the leaves sprayed with cuprous oxide-cotton-seed oil emulsion. A comparison with Table VIII B indicates the superior fungicidal value of the cotton-seed oil-Bordeaux mixture on both leached and unleached leaves.

(iii) *Comparison of results with data from chemical estimates*

From the results given in Tables IV–IX it would appear that the following materials had no adverse effect on tenacity in the experiments described—Agral 2, gelatine, lime casein, sulphonated lorol and cotton-seed oil. Sulphite lye had a markedly deleterious effect on tenacity and this effect was exhibited, to a smaller extent, by emulsions of white oil and of cotton-seed oil having sulphite lye as the emulsifier. Finally, in a single experiment, a slightly adverse effect on tenacity was shown by methyl cellulose.

For comparison of these findings with the data on tenacity determined by chemical estimation, an extract is given in Table X from the results obtained by Fajans & Martin (1937). The figures concerning the effects of supplements on the tenacity of cuprous iodide are given for the concentrations equal to those used in the biological tests.

Table X

Tenacity of cuprous iodide on a cellulose nitrate surface

Figures extracted from Fajans & Martin's Table III, 1937.

Spray supplement	Concentration %	Tenacity %
Gelatine	0·05	88
Petroleum oil emulsion	1	78
Agral 2	0·05	61
Nil	–	28
Methyl cellulose	0·05	23
Sulphonated lorol	0·05	14
Sulphite lye	0·5	2

The biological data are seen to agree generally with the findings of Fajans & Martin concerning the effect on tenacity of gelatine, Agral 2, methyl cellulose and sulphite lye. The adverse effect of sulphonated lorol, however, has not been demonstrated in a biological test, and the effects of petroleum oil emulsion, as given in Table VII, have been predominantly unfavourable to tenacity. This point is referred to further below. It should be noted, however, that in the present investigation no tests were made in which the petroleum oil emulsion was used in conjunction with cuprous iodide and cuprous oxide as in the experiments of Fajans & Martin.

The results given in Tables VIII A and VIII B showing that cottonseed oil alone has no adverse effect on tenacity are in agreement with a number of chemical findings that this oil acts as a sticker (see Martin, 1933).

(iv) *Comparison of results with data from field trials*

(1) *Cuprous cyanide.*

The results recorded in Table III show that cuprous cyanide at 0·2% copper retained some fungicidal value on leaves but was inferior to Bordeaux mixture of the same copper concentration. An account of a field trial using cuprous cyanide against Pear Scab is given by Marsh *et al.* (1937). A summary of the results obtained is given in Table XI.

These results suggest that cuprous cyanide had proved slightly inferior to the Bordeaux mixture. It might be anticipated from the

results of the laboratory leaf test that this inferiority would be even greater than was shown in the field trial.

Table XI

Results of field trial against Pear Scab

Spray	Total fruits	Clean %	Slightly scabbed %	Badly scabbed %
Bordeaux mixture 0·1% Cu	1653	24	75·5	0·5
Cuprous cyanide 0·12% Cu	1219	19	80	1
No spray	404	0	79	21

(2) *Effects of spray supplements on tenacity.*

In the field trials listed below, the spray supplements have in general been used with lime sulphur, and their effects on tenacity have been gauged by the control obtained of Apple Scab. The full details of the experiments are to be found in the Long Ashton *Annual Reports* of the appropriate years unless otherwise stated.

(*a*) *Sulphonated lorol.* The effect of a 0·05% sulphonated lorol supplement to a lime sulphur spray programme is reflected in the figures given in Table XII on the control of apple scab.

Table XII

Percentage scab-free fruit obtained in field trial at Long Ashton, 1936

Variety	Spray treatment: Unsprayed	Lime sulphur	Lime sulphur + sulphonated lorol
Newton	86	99	94
Lane	83	99	99
Bramley	81	98	99
Allington	79	97	98
Cox	75	99	98
Worcester	68	81	85

It will be noted that the addition of the sulphonated lorol has not brought about any reduction in the fungicidal effect of the lime sulphur, thus corroborating the result given in Table VI.

(*b*) *Agral* 2. No figures are available concerning the effect of Agral 2 on tenacity in field trials but, in a number of experiments on apple sawfly control when this supplement has been used with lime sulphur and an insecticide, no reduction in the standard of scab control has been observed.

(*c*) *Petroleum oil-sulphite lye emulsion.* The records concerning the effect of petroleum oil-sulphite lye emulsions are assembled in Table XII. The results dated 1937, not previously published, relate to a post blossom

Table XIII

Results of field trials against apple scab using petroleum oil-sulphite lye emulsions as spray supplement

Locality	Date	Variety	Lime sulphur concentration %	Oil emulsion concentration %	Results: With lime sulphur alone: Total fruits	Results: With lime sulphur alone: % scab-free	Results: With lime sulphur + emulsion: Total fruits	Results: With lime sulphur + emulsion: % scab-free
Hereford	1935	Derby	3	7½	5,106	95·0	7,503	95·6
,,	1935	Rival	3	7½	759	96·8	692	96·4
Long Ashton	1936	Allington	3, 3, 1*	7½, 4½, 1*	1,384	97·0	956	98·0
,,	1936	Bramley	3, 3, 1*	7½, 4½, 1*	956	98·0	2,128	79·5
,,	1936	Cox	3, 3, 1*	7½, 4½, 1*	1,256	98·6	728	97·4
,,	1936	Lane	3, 3, 1*	7½, 4½, 1*	888	99·0	644	97·2
,,	1936	Newton	3, 3, 1*	7½, 4½, 1*	296	98·3	472	97·3
,,	1936	Worcester	3, 3, 1*	7½, 4½, 1*	912	80·7	2,228	77·1
Isle of Ely	1936	Bramley	3, 1, 1	4½, 1½, 1½	689	53·8	1,216	57·3
Long Ashton	1937	Worcester	1†	1	29,689	55·8	23,764	54·1

* In successive sprayings.

† 1% lime sulphur + 0·05% sulphonated lorol.

spray on two blocks of Worcester Pearmain apples at Long Ashton which had been treated alike in the earlier sprayings.

The trials listed in Table XIII have covered a range of varieties, concentrations, degrees of scab attack and localities. In one instance, on the trees of Bramley at Long Ashton in 1936, there is an indication that the presence of the oil emulsion has lowered the level of scab control. Otherwise, the results obtained agree in showing that the oil emulsion neither increases nor decreases the fungicidal value of the lime sulphur deposit. This contradicts the conclusion drawn from Table VII that the effect of the petroleum oil-sulphite lye emulsion would be to lower tenacity.

(*d*) *Cotton-seed oil emulsion.* Table XIV gives the results of a field trial carried out on Williams' pears at Long Ashton in 1937 using the same materials and concentrations as employed for the laboratory leaf tests recorded in Table IX. These sprays were (1) a paste preparation of cuprous oxide compounded with a cotton-seed oil emulsified with sulphite lye, used at a concentration of 0·1% copper and 0·025% cotton-seed oil: (2) a cotton-seed oil-Bordeaux mixture used at the same copper and cotton-seed oil concentrations as (1). The sprays were applied to comparable blocks of trees twice before blossoming and once after. Their fungicidal value was assessed by counts of scabbed and of clean fruits made in late August.

Table XIV

Results of field trial on pear scab control using cotton-seed oil

Treatment	Total fruits	Scab free %	Lightly scabbed %	Badly scabbed %
Cuprous oxide-oil	849	19	53	28
Bordeaux-oil	570	33	43	24
No spray	421	1	23	76

It is seen that both treatments gave fair scab control, but the advantage of the Bordeaux spray over the cuprous oxide was not significant. In comparison with the Bordeaux-oil, the cuprous oxide-oil spray appeared to greater advantage in the field than in the laboratory leaf tests.

Discussion

The value of a material for use as a protectant fungicide is judged from the fungicidal effect of the dispersed residue remaining after dilution and drying. If this residue is deposited on a non-living surface such as a glass slide its retention and tenacity will be governed only by physical

factors and, with suitable precautions, a standardized method of presenting the fungicide is possible. The biological criterion of fungicidal value in general use is the effect of this deposited residue in inhibiting the germination of fungus spores. The resistance to fungicidal effect shown by spores placed in suspension on the deposit is subject to modification by such factors as temperature, age of the spores, concentration in the drop of suspension and presence of nutrient materials in the drop. The method described by Montgomery & Moore (1938) of using washed spores from cultures of standard age, suspended evenly in distilled water, gives biological testing material of a high degree of uniformity.

In the present investigation, certain departures from these levels of standardization have been made. The spores employed have been taken from naturally occurring infections in the field and have undoubtedly carried small quantities of nutrient material. This factor decreases sensitivity to fungicides and is of importance in considering the differences between the results given above in Table I and those published by Montgomery & Moore (1938). Tests have also been carried out on leaf surfaces, which again introduce variable factors having the general effect, when compared with tests *in vitro*, of necessitating an increase in the concentration of a fungicide required to inhibit spore germination. The motive in employing these methods of testing has been to explore the possibility of determining by laboratory methods the approximate strengths of fungicides required for use under field conditions.

In this connexion, special consideration has been given to the use of the laboratory leaf testing method for appraising the merits of materials put forward for use as protectant sprays against apple and pear scab. In other words, the test is directed towards evaluating the resultant of the complex series of factors determining one type of field performance of a fungicide. Each of these factors must be held constant at some arbitrary level in a laboratory test, and the value of the method can be judged only by the measure of concordance of the results with those of a comparable series of field trials. When the relation of results from a laboratory test to those from field trials is established, the test can be assigned its place in the assaying of new spray materials.

Considering in turn the factors determining protectant value of a fungicide, the factor of evenness of distribution is easily kept constant in the laboratory while, in the field, it will be adversely affected not only by variations in spray application but, also, by the persistent growth of new tissue subsequent to spraying. The amount of initial retention of a fungicide is commonly determined by the spray supplement employed

and the nature of the surface sprayed. In laboratory tests spray retention can be held constant by limiting the period of application so that the point of run-off is not reached. Where a number of wetters is being compared, and it is desired to keep the retention of fungicide constant, the amount applied must be determined by the most surface-active wetter and will be much below the run-off figure for many of the other supplements. In determining tenacity, the time, period and method of leaching used in the laboratory are all arbitrary. If resistance to leaching rises after 24 hr., the fungicide in the field would commonly have one advantage over that in the laboratory, since the likelihood is that the period between spray application and rainfall would be more than a day.

In testing availability of the spray residue in the laboratory, the approximation to field conditions is made by using the same fungus spores and leaf surfaces. In evaluating toxicity, the concentration of spores employed in the laboratory is commonly greater per unit area than would be encountered in the field, and the spores are given optimum conditions for germination. Further, it cannot be assumed that, in the field, infection in all circumstances follows germination. In these respects the conditions in the laboratory tests, as compared with those in the field, are biased against the fungicide.

Taking first the results obtained on fungicides without supplements, there is a fair measure of concordance between the results of laboratory leaf tests and field performance. Lime sulphur concentrations of 1%, and concentrations of Bordeaux mixture, cuprous oxide and cuprous cyanide equivalent to 0·1–0·2% copper, which have shown a generally high level of fungicidal value in the laboratory leaf tests, are in fact the concentrations actually effective for scab control in the field. The tendency is for the laboratory leaf tests to be somewhat less favourable to the fungicide than the field results.

This tendency is shown more markedly in the tests dealing with the effects of supplements on tenacity of fungicidal residues. An example of special interest is the effect of the petroleum oil-sulphite lye emulsion, since this product is frequently employed with insecticides in apple spraying as a supplement to lime sulphur. In the laboratory leaf tests, the effect of the emulsion was to reduce the fungicidal value of the lime sulphur deposit but in field tests it generally showed no such adverse effect.

It is suggested that this apparent discordance of results is related to the fact that the maximum possible initial retention of a spray including petroleum oil emulsion is higher than that of sprays containing wetting agents such as Agral 2 or sulphonated lorol (see Fajans & Martin, 1937).

In the laboratory tests, spray retention was kept low throughout so that the effect of a number of supplements could be compared using a constant deposition of fungicide. In the field, on the other hand, spray application is not stopped until the region of run-off is reached so that the initial deposition will, here, approach the maximum retention figure. The leaching process in the laboratory is thus applied to a much smaller quantity of fungicide per unit area than in the field. In both instances the presence of the emulsion will lead to some loss of tenacity but, in the field, it appears that the amount of spray residue initially present is sufficiently in excess of that required for protectant value to sustain this loss without perceptible reduction in fungicidal effect.

From the results obtained in the present investigation it would appear that the laboratory leaf test, carried out as described, provides a useful method of rapidly appraising the value of a fungicide for use against apple or pear scab. Any material which passes this test successfully is likely to merit field trial at a concentration equal to or lower than that employed in the laboratory. The experience in testing combined sprays suggests that with these mixtures it would be preferable to carry out a series of tenacity tests on deposits ranging in amount up to the level of maximum retention on the leaf surface.

SUMMARY

1. Laboratory sorting-out tests on glass slides have shown the relative fungicidal value of a number of rubber accelerators and other organic sulphur derivatives. The most toxic of these materials, when tested on leaves, gave no promise of being of use in the field.

2. In laboratory leaf tests using spray supplements the adverse influence of sulphite lye on tenacity was illustrated by spore germination experiments. Loss of tenacity was similarly demonstrated in sprays including oils emulsified with sulphite lye.

3. A limited number of comparisons with field trials indicates that the laboratory leaf test may be used as an indicator of the fungicidal value of a spray material against *Venturia inaequalis* and *V. pirina*, but that in general it gives results less favourable to the fungicide than those from corresponding field trials.

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THE TOXICITY OF ETHYLENE OXIDE TO *CALANDRA ORYZAE*, *C. GRANARIA*, *TRIBOLIUM CASTAÑEUM*, AND *CIMEX LECTULARIUS*

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(With 11 Text-figures)

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Introduction

The growing importance of fumigation as a method of controlling insect pests calls for further accurate knowledge both of a particular and general nature. The former is concerned with the resistance of specific pests to various gases, while the latter deals with toxicological relationships.

Fumigation research is a comparatively new science demanding special theory and technique; but these should be developed in accordance with general physiological methods.

The object of this paper is twofold: it presents the results of investigations into the toxicity of ethylene oxide to common insect pests; also the relationships between concentration, time of exposure and mortality are investigated and compared with analogous work.

This research is essentially experimental and quantitative. The experimental method implies variation of one condition while others remain constant. The quantitative qualification means measurement and statistical treatment of the results. Before describing the experimental procedure it is proposed to survey the precautions necessary for efficient fumigation research. A considerable volume of published data is rendered unreliable through inaccurate measurement of variables and failure to maintain constant conditions. The points to be considered are:

(1) *Constant factors*:
- (*a*) Species of insect.
- (*b*) Fumigant.

(2) *Variable factors*:
- (*a*) Those influencing the amount of poison absorbed:
  - (i) Concentration.
  - (ii) Exposure time.
  - (iii) Temperature.
- (*b*) Those influencing physiological poisoning processes:
  - (i) Temperature.
  - (ii) Humidity.
  - (iii) Carbon dioxide and oxygen concentration.
  - (iv) "Pre-fumigation" effect.
  - (v) Age and stage of the insect.
  - (vi) Starvation.

(1) *Constant factors*

Species of insect and type of fumigant.

The resistance of different species and genera of insects to a given fumigant may be extremely different. Cotton (1932) has pointed out that even two species of the same genus (*Calandra granaria* and *C. oryzae*) are quite different in susceptibility to ethylene oxide.

Experience in working with one series of insects and several fumigants has revealed another interesting fact, namely, that the order of resistance may change from one poison to another (Table I).

The remarkable changes of relative resistance of the insects mentioned in Table I cannot, at present, be explained. A clue, however, is offered in the widely different effects on the behaviour of the insects caused by the three fumigants. Hydrogen cyanide causes stupor in about a minute even in low, non-lethal doses. Sulphur dioxide in severe and fatal doses causes stupor after an hour or so, while ethylene oxide

has usually no apparent effect until death occurs several days after fumigation. Possibly, with further knowledge of this kind, one may be able to classify gases by the order of resistance of species and by their effects, since presumably both depend on their mode of action. At present the main importance of the matter lies in the practical fact that caution must be observed in arguing from the results of one fumigant to another or from one insect to another.

Table I

Relative resistance of four insects to three fumigants

Fumigant	Species	Temp. °C.	5-hr. lethal concentrations (mg./l.) N.T.P.	Author
Hydrogen cyanide	*Calandra granaria*	25	14·00	Peters & Ganter (1935*a*)
	C. oryzae		12·00	Allison (1928)
	Tribolium castaneum		0·36	Bovingdon (1935)
	Cimex lectularius		0·17	Bovingdon & Busvine (1936)
Ethylene oxide	*Tribolium castaneum*	25	27·00	This paper (99% lethal dose)
	Cimex lectularius		12·30	
	Calandra granaria		8·40	
	C. oryzae		4·10	
Sulphur dioxide	*Calandra oryzae*	20	10·80	Busvine (1936)
	Tribolium castaneum		9·70	
	Calandra granaria		8·30	
	Cimex lectularius		5·90	

(2) *Variable factors*

(*a*) *Influencing the amount of poison absorbed.*

Insects are dosed with fumigant poisons by the simple method of subjecting them to a constant concentration for a definite time. The maintenance of constant physical conditions has been achieved in this work by the use of the fumigation apparatus designed and described by Bovingdon (1934) and the difficulties in accurate measurement of small quantities of ethylene oxide were largely overcome by the technique developed by Lubatti (1932, 1935).

(i) *Concentration*. The maintenance of a constant concentration of gas over experimental insects presents several difficulties. In large fumigation chambers it is often found that the concentration falls considerably during the experiment because of leakage and absorption. In smaller glass vessels the difficulty of accurate dosage is increased, and there is still the possibility of bad distribution of gas unless adequate stirring devices are installed. Experiments with sulphur dioxide and ethylene oxide may be quoted to illustrate this (Tables II and III).

Table II

Gas: Sulphur dioxide. Vessels: 7–8 l. desiccator. Temperature: 20° C. Method of stirring: Swinging suspended card. Method of sampling: Capillary to evacuated flask containing iodine solution. Titration against thiosulphate.

Sample after (min.)	% expected concentration found			
	Top	Middle	Bottom	
1–2	29·5	97·5	236	Not stirred
8	75·5	97·0	143	,, ,,
65	88·5	96·0	96	,, ,,
2–3	95·5	97·5	104	Stirred
2–3		100·5		,,
2–3		97·0		,,
2–3		98·5		,,
		Av. = 98·5		

In order to eliminate the possibilities of error from bad distribution of gas, the ethylene oxide-air mixture was stirred about fifty times before the commencement of each experiment. Inspection of Table III shows that this should be ample.

Table III

Gas: Ethylene oxide. Vessel: Flask of Bovingdon's apparatus (4 l.). Temperature: 25° C. Method of stirring: Agitation of a plunger. Method of sampling: Evacuated flask to sampling connexion. Estimation by Lubatti's technique.

Sample after min.	Number of stirs	% expected concentration found
10	0	430
30	0	214
40	0	194
230	0	156
10	0	430
10	5	178
10	12	114
10	25	100
10	50	100
10	75	102

In order to keep a check on possible leakage and to increase accuracy of dose estimation, a sample was taken at the beginning and end of each experiment.

The comparatively small size of the fumigation flask in Bovingdon's apparatus (about 4 l.) made it possible to draw only small samples for analysis. Accordingly flasks of about 130 c.c. were used, each containing a standard quantity of magnesium bromide-sulphuric acid absorbent and provided with a capillary tube with a tap. The flasks were evacuated to a known pressure, and connected to the sampling connexion of the apparatus by the capillary tube. The tap was then opened and the flask

left in connexion with the apparatus for 5 min. to allow the gas drawn to reach room temperature. Titration was done about an hour after drawing the sample. A number of check experiments showed that if a very short time was allowed, the absorption was not complete. On the other hand, with a long delay there was a tendency for the absorbent to weaken by loss of hydrogen bromide. The titration was done with 0·05 N solution of sodium hydroxide in a micro-burette and comparison with a blank indicated the amount of absorbent which had reacted with ethylene oxide. From this, the concentration of the gas was calculated and reduced to N.T.P.

The concentrations measured ranged from about 1 to 100 mg./l. The estimated accuracy in determination amounted to 0·30 mg./l. This corresponds to an error of 30% at 1 mg./l., and 0·30% at 100 mg./l. The large error was to some extent reduced by taking the average of three determinations when the doses were low. These low concentrations occurred only in the 20 hr. exposures.

As a general rule very little variation in adjacent samples was observed. There was no appreciable tendency to leakage, and the circulating and stirring devices in the apparatus maintained good gas distribution in the main flask. The average difference in the usual two samples was about 3%.

(ii) *Time of exposure.* The time of exposure in all experiments was measured simply by wrist watch. The inaccuracy involved in measurement was practically negligible since an error of ½ min. amounts to only 1·66% with the shortest exposure. The difficulty of measuring exposure time is due to the fact that the insects cannot be exposed to the full concentration instantaneously.

The 60 c.c. animal chamber of Bovingdon's apparatus was flushed through with twice its volume of air-gas mixture from the reserve flask. It was then turned into communication with the main flask and the circulation pump started. Even with these precautions, there is a definite lag before full experimental concentration is attained. This is shown by Table IV, which gives results of special test samples taken directly from the animal chamber.

Table IV

Concentration in the animal chamber

Period before sample (min.)	0	5	10	20
% final concentration	78	87·8*	94·5*	98

* Average of two experiments.

By integrating a curve based on Table IV, the effect of the lag may be estimated as being equivalent to a complete hiatus of 1·6 min. (assuming concentration × time for a given effect to be constant).

(iii) *Temperature and its effect on physical constants.* With high boiling point vapour poisons (e.g. orthodichlorobenzene B.P. = 176° C.), a rise in temperature even within the biological range may increase considerably the vapour pressure. As a result the saturation concentration becomes greater, and higher doses become available. This does not apply to low boiling point fumigants such as ethylene oxide, hydrogen cyanide or sulphur dioxide since saturation concentrations are never approached.

Two other physical properties, influenced by temperature, are concerned with poison uptake. With a rise in temperature the rate of *diffusion* of gases is increased while *sorption* is decreased. The temperature coefficients of diffusion of gases are extremely close to unity. Those dealing with solution and adsorption of gases are almost invariably fractional. The values of Q_{10} for $\frac{1}{\text{lethal dose}}$ at different temperatures,[1] on the other hand, are usually between 1·5 and 5 (see Table VI). It seems, therefore, that in so far as the effect of temperature is concerned neither diffusion of gas in air nor absorption by the insect plays a predominating part in regulating the rate of poisoning of insects by fumigants. (These observations refer only to experimental conditions in which the insects are surrounded by a standard concentration of gas. In these circumstances diffusion only refers to passage of gas along the tracheae of the insect.)

The effects of temperature on toxicity appear to be bound up with chemical poisoning processes and also with the physiology of the insect which will be considered later.

(*b*) *Factors influencing the poisoning process.*

The action of a poison is to dislocate the normal metabolism of an organism and, therefore, it is not surprising that the degree of poisoning is closely bound up with the physiological condition of the organism. For this reason chemical methods of pest control would benefit by elucidation of certain physiological aspects of biology which have been comparatively neglected in the past.

The salient points in the relationship between the physiological condition of insects and their resistance to poisons can best be illustrated by means of a table (Table V).

[1] The provisional use of Van't Hoff's coefficient in this connexion is justified on p. 611. The introduction of a more valid temperature coefficient awaits a more thorough understanding of toxicological processes.

Table V

Physiological condition and resistance

Cause	Physiological effect	Toxicological effect
Raising temperature	Increases metabolism (e.g. respiration): Vernon (1897), Crozier (1924), Batelli & Stern (1913), Sayle (1928), Krogh (1914), Rodgers (1929), Bodine (1921)	Increases susceptibility: Jones (1933), Cotton (1932), Peters & Ganter (1935*a*), Bovingdon & Busvine (1936)
Varying relative humidity	No pronounced effect on metabolism: Rivnay (1932), Mellanby (1936)	No pronounced effect on susceptibility: Brinley & Baker (1927), Lindgren & Shepard (1932)
Addition of carbon dioxide	Increases respiratory movements: McGovran (1932). Opens spiracles of insects: Hazelhoff (1927), Wigglesworth (1935)	Increases susceptibility to non-stupefying gases: Cotton & Young (1929), Pratt, Swain & Eldred (1933)
Lowering oxygen content of the air	Increases respiratory movements: v. Buddenbrock & Rohr (1923), Babak & Foustka (1907). Opens spiracles more often: Wigglesworth (1935)	Increases susceptibility (to various gases): Cotton (1932)
Small preliminary dose of hydrogen cyanide	Lowers respiratory rate and causes stupor: Buchanan (1926), Dixon & Elliot (1929), Child (1919), Hyman (1916), Shafer (1911), Allen (1911)	Decreases susceptibility (to hydrogen cyanide): Gray & Kirkpatrick (1929), Pratt, Swain & Eldred (1931)
Age and stage of insect	Order of respiratory rate: adult → larva → pupa: Batelli & Stern (1913), Ludwig (1931)	Order of susceptibility: adult → larva → pupa: Cotton (1932)
Starvation	Respiration rate and R.Q. decrease: Fink (1925), Child (1919), Cook (1932), Bodine (1921)	Susceptibility to ethylene oxide decreases: Mayer (1934) and this paper Susceptibility to hydrogen cyanide increases: Bovingdon & Busvine (1936)

The purpose of this work is to deal mainly with the relationship between mortality, dose and time, other factors being constant. However, it will be necessary to consider briefly the importance of the various influences of metabolism and to assess the efficiency with which they have been stabilized.

(i) *Temperature*. The fact that the resistance of insects decreases with a rise in temperature is well known, but very little accurate work has been done to determine the magnitude of the effect. The quantitative effect on normal physiological processes has been referred to three types of formula:

(1) According to Krogh (1914) the relation is linear within the normal biological range.

(2) Van't Hoff's equation $\frac{\text{Rate at } t+10}{\text{Rate at } t} = \frac{Q}{10}$ has been widely used, but the value Q_{10} is not a constant. It usually decreases from about 4 at 10° C. to about 1·5 at 30° C.

(3) Arrhenius has proposed a formula:

$$\frac{V_1}{V_0} = e^{\frac{\mu}{2} \frac{(T_1 - T_0)}{(T_1 T_0)}}$$

(V_0 = velocity at temperature T_0, V_1 = velocity at temperature T_1), which allows for this fall in Q_{10}. However, two or three values of his constant μ are usually required to fit different portions of the temperature range.

Neither the linear relation nor Arrhenius's formula expresses the relation between temperature and toxicity. The effect can be expressed provisionally by values of Q_{10} corresponding to Van't Hoff's coefficient, which is convenient because of general use.

In place of velocity $\left(=\frac{1}{\text{time}}\right)$, the toxicity can be measured as $\frac{1}{\text{concentration}}$ for a predetermined kill. The increase in toxicity corresponding to a 10° rise in temperature can be calculated for any results at two or more temperatures from the formula:

$$Q_{10} = \left(\frac{C_1}{C_2}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

(C_1 = lethal concentration at T_1, C_2 = lethal concentration at T_2).

Table VI shows values calculated in this way from data of different workers. These results show an unfortunate lack of uniformity, which makes it impossible to generalize.

That this is not due to different experimental methods or different insects is shown by the great difference in the Q_{10} values of ethylene oxide and hydrogen cyanide towards *Calandra* obtained by the same technique. Such discrepancies are reminiscent of the conclusions of Hartmann (1918), who, working with *Cladocera* in toxic solutions, found no uniformity in temperature coefficients and concluded that their changes in value indicated different complex processes (e.g. osmotic pressure, ionization etc.) underlying the toxic action of different poisons. The various processes are probably dominant to different extents according to the position on the temperature scale.

The present series of experiments were all done at 25° C. From the limited evidence available, the value of Q_{10} would be expected to be about 2·5. The variation in the temperature of the fumigation cabinet did not exceed ±0·15° C. (Bovingdon, 1934). On the above assumption this corresponds to a dose error of ±1·4%.

(ii) *Relative humidity.* The work of Lindgren & Shepard (1932)

Table VI

Values of Q_{10} for fumigants

Authors	Insect	Fumigant	Exposure range hr.	Temperature range (°C.) 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 — Values of Q_{10}
Wilmot & Walker (1932)	*Mineda juglandis*	Hydrogen cyanide	½	← 3·24 →
Cotton (1932)	*Tribolium confusum*	Ethylene oxide	3	← 3·33 →← 3·46 →
Jones (1933)	*Limonius* spp.	Carbon disulphide	5	← 1·2 →← 1·72 →← 1·78 →← 2·03 →← 1·31 →
Peters & Ganter (1935*a*)	*Calandra granaria*	Hydrogen cyanide	2½–30	← 0·48 →← 1·04 →← 1·59 →← 1·88 →← 2·18 →
Peters & Ganter (1935*b*)	*C. granaria*	Ethylene oxide	2½–30	← 5·18 →← 2·40 →← 1·95 →← 1·11 →
Bovingdon & Busvine (1936)	*Cimex lectularius*	Hydrogen cyanide	½–2½	← 2·7 →← 4·7 →
Parkin & Busvine (1937)	*Lyctus* sp.	Hydrogen cyanide	1–6	← 2·47 →
Lindgren (1935)	*Tribolium castaneum*	Carbon disulphide	5	← 3·2 → ← 1·28 → ← 1·45 →← 2·05 →← 1·90 →
		Chlorpicrin	5	← 2·63 → ← 1·45 → ← 1·80 →← 2·47 →← 2·42 →

suggests that, over a wide range, relative humidity has little effect upon resistance of insects to fumigants.

In all the experiments carried out the air was conditioned to about 45% R.H. by passing it through solutions of potassium hydroxide. The amount of variation, observed by an "Edney" paper hygrometer, was well within ±5% and therefore could be neglected.

(iii) *Influence of carbon dioxide and oxygen pressure.* Neither effect has been studied in this work and their influence is excluded by using normal air deprived of all carbon dioxide by the conditioning potash solutions.

(iv) "*Prefumigation*" *effect.* Since ethylene oxide does not cause stupefaction, the possibility of small doses causing a fall in the rate of metabolism is unlikely and the design of Bovingdon's apparatus is such that the insects are exposed to almost the full concentration of the gas at the commencement of the experiment.

(v) *Influence of age and stage of insect.* The close dependence between rate of metabolism and susceptibility is supported by indirect evidence provided by the different stages of insect as pointed out by Cotton (1932). The order of metabolic rate: adult → larva → pupa has been paralleled by a similar order of susceptibility.

There are, however, a few exceptions which indicate that other factors besides metabolic rate come into question. For example, *Lyctus* larvae are apparently more susceptible than the adults (Parkin & Busvine, 1937). Also work on the bed-bug has shown that the order of susceptibility to ethylene oxide, hydrogen cyanide and orthodichlorobenzene is: eggs → young nymphs → adults → 4th and 5th stage nymphs, but towards sulphur dioxide, and tri- and perchlorethylene, the eggs are about twice as resistant as the adults and old nymphs.

In the present series of experiments only adults of the stored product pests were used and only 4th and 5th nymphal bed-bugs. Apart from this no special precautions were taken to ensure that the insects were exactly of the same age. The beetles were chosen at random from healthy cultures.

(vi) *Starvation.* The effect of starvation was only studied in *Cimex* where it is of practical importance. Bugs can endure long periods of starvation and take relatively enormous meals of blood, and it is not surprising that their resistance varies at different times.

Their resistance to hydrogen cyanide decreases from the first day after feeding, though immediately after the meal it is low, probably because of the large amount of water in the food which may absorb the soluble gas.

The relation between starvation and resistance of bugs to ethylene oxide is entirely different, for, as pointed out by Mayer (1934), starved bugs are more resistant than recently fed ones. This is, perhaps, due to the decreased rate of metabolism which seems to occur in starving invertebrates (Fink (1925), *Leptinotarsa* and Bodine (1921), locusts). The different effect of hydrogen cyanide recalls the fact that this gas is exceptional in not being influenced by addition of carbon dioxide (Cotton, 1932). These anomalies may be due to the sudden stupefying action of hydrogen cyanide which probably obscures minor influences upon metabolism.

The enhanced rpsistance to ethylene oxide does not increase in a linear fashion. Fig. 1, which shows medium lethal doses (calculated

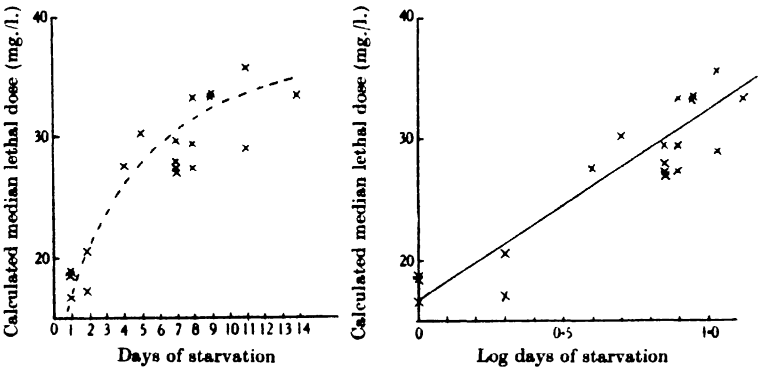

Fig. 1. Resistance of *Cimex* after different periods of starvation

from a number of experiments and corrected for control mortality) plotted against the logarithm of the starvation period, suggests that the relationship is logarithmic.

For practical purposes resistance may be said to be highest 7–12 days after starvation at 25° C. and bugs in this condition have been used almost exclusively in the present experiments. After about 14 days the deaths from starvation more than compensate for the enhanced resistance.

Experimental procedure

(1) *Planning of experiments*

(*a*) *Arrangement.*

The experiments with ethylene oxide were planned to provide concentration-mortality regression lines for each insect at a number of different exposures. Data obtained in this way were intended to show

time-concentration relationships for a constant effect (mortality). It was expected that the relation would be of the form:

$$c^n t = K,$$

(c = concentration, t = time, c and K = constants),

or

$$n \log c + \log t = \log K.$$

Therefore the exposure times were chosen, so far as was practicable, at constant logarithmic intervals. The selected times were ½, 1, 2, 4, 6, 9 and 20 hr.

(*b*) *Accuracy.*

In order to gather greatest information about time-concentration relationships it is desirable to obtain data over as wide a range as possible but, unfortunately, at either extremity of the curve a decrease in accuracy sets a limit to useful exploration. This is illustrated by Table VII. With long exposures the concentrations are low and the percentage error of estimation becomes high. The accuracy was to some extent improved, however, by taking the average of two and sometimes three determinations for each experiment. The only way to obtain uniform accuracy throughout would be by carrying out many more long exposure experiments and this was not found practicable.

The limitation of the short exposures was not due to a reading error which, as explained before, is negligible. The difficulty here is due to the lag in attaining full concentration. From calculations based on Table IV (assuming $c \times t \simeq$ constant) the lag is approximately equivalent to a complete hiatus of 1·6 min. This is about 5% of a half-hour exposure, and it was decided that shorter exposures would involve too great and too uncertain an error. A correction was not introduced, since it would involve too many assumptions.

Table VII

Percentage accuracy of time and concentration

Estimated accuracy of measurement		Exposure time (hr.) ½	1	2	4	6	20
1/3 mg./l.	% M.L.D. of *Tribolium*	0·56	0·71	1·30	2·12	2·56	6·15
	% M.L.D. of *C. granaria*	0·92	1·92	3·06	5·80	8·4	19·2
	% M.L.D. of *C. oryzae*	1·25	2·75	5·6	10·6	13	56
1·6 min. lag	% time of exposure	5·3	2·67	1·34	0·67	0·45	0·13

M.L.D. = median lethal dose.

The experimental range is, therefore, limited by the lag in exposures shorter than ½ hr. and the low concentrations found in exposures greater

than 20 hr. Within this range the accuracy is variable, but this will find expression in the standard error of individual regression lines and can be allowed for in a statistical analysis of the results.

(2) *Treatment of insects*

The stored product insects were taken from cultures in 7 lb. jars containing appropriate food (grain for *Calandra granaria*, rice for *C. oryzae* and wholemeal flour for *Tribolium*). New cultures were used whenever signs of overcrowding, moulds, or mites were observed.

The bed-bugs were kept in muslin-topped glass tubes and fed once a week on the ears of a lop-eared rabbit.

All the insects were reared at 25° C. and about thirty insects were used in each experiment. They were exposed to the fumigant in small muslin bags and afterwards transferred to clean glass tubes. Suitable food was added and the tubes were corked and kept in an incubator at 25° C. A number of special experiments demonstrated that corking the tubes after fumigation did not have any harmful effects.

(3) *Estimation of mortality*

There are several difficulties in ascertaining mortality of insects after fumigation and these can only be overcome by periodic observations over a considerable period following the experiment. The primary difficulty is to distinguish which insects will finally succumb. In the first place the toxicological effect is graded and not absolute as every state can usually be found between slightly affected to moribund and dead insects. The affected insects sometimes remain partially paralysed for long periods before final death or recovery. A further difficulty is introduced by the stupefying effects of some gases. Immobility may be caused almost at once (hydrogen cyanide) or after about an hour (orthodichlorobenzene, etc.) and the duration of the stupor may be a matter of hours or even extend to weeks. The period of greatest rate of revival of bed-bugs after exposure to hydrogen cyanide or orthodichlorobenzene is 2–4 days after the experiment (at 25° C.). After about 7–10 days, revival of the great majority of bugs has occurred. *Lyctus* larvae on the other hand have been observed to recover after 17 days at 25° C. and 21 days at 20° C. during which they showed no signs of life (Parkin & Busvine, 1937).

The rate of revival is related to the severity of the exposure. If experiments are grouped according to the percentage of recovery it

is evident that the more severely affected insects (85–99% kill) do not recover as quickly as less heavily dosed ones (70–84% kill) (Table VIII).

Table VIII

Rate of revival of bed-bugs after fumigation with HCN at 25° C. Expressed as the percentage of maximum revival in each class

Mortality range of experiments	Days after experiment 1	2	3	4	5	6	7	8	9	10	11	12	
85–99%	4	5	—	27	—	75	—	100	—	100	—	94	% max.
70–84%	28	—	40	—	80	94	100	—	100	—	91	-	revival

Ethylene oxide introduces the problem of a delayed death in place of a partial revival. The mortalities in the present work were estimated in each experiment when the rate of deaths had decreased to the normal low rates observed in controls. (*Calandra oryzae* and *C. granaria* 2–4% and *Tribolium castaneum* about 5% in 7–10 days.)

The rate with *Cimex* constitutes an exception owing, no doubt, to the fact that bugs were not fed for at least a week before the experiment nor during the subsequent period of examination. The control deaths amounted to approximately 1% after a week's starvation, 9% after 2 weeks and 21% after 3 weeks. A correction was made by estimating the true kill by Abbott's formula:

$$\text{True kill} = \frac{100\,(x-y)}{100-y},$$

where x = observed % kill and y = % control deaths.

The correction was adjusted in each experiment according to the total period of starvation.

The duration of the interval before death is related to the severity of exposure, the greatest rate of death occurring earlier with more

Table IX

Percentages of final mortalities on different days after fumigation with ethylene oxide

Insect	Mortality range %	Days after the experiment 1	2	3	4	5	6	7	8	9	10	11
Calandra oryzae	1–49	—	9	40·0	59	67	80·5	80·5	95	97	100	100
	50–99	—	69	79·0	84	92	95·0	96·0	94	99	100	—
C. granaria	1–49	—	44	72·0	68	90	93·5	95·5	96	97	100	100
	50–99	—	77	79·5	93	91	96·0	97·0	99	100	100	
Cimex lectularius	1–49	-	63	75·0	91	94	97·0	97·0	100	100	—	
	50–99	-	90	95·0	97	98	99·0	99·0	100	- -	—	—
Tribolium castaneum	1–49	60	89	100·0	89	100	—	—	—	-	—	-
	50–99	91	100	100·0	—	—	—	—	—	—	—	—

severely dosed individuals. (This is the converse of the relationship obtaining with revival from stupefying gases.)

The length of the delay also depends upon the species of insect. Both these facts are illustrated by the analysis of mortality presented in Table IX.

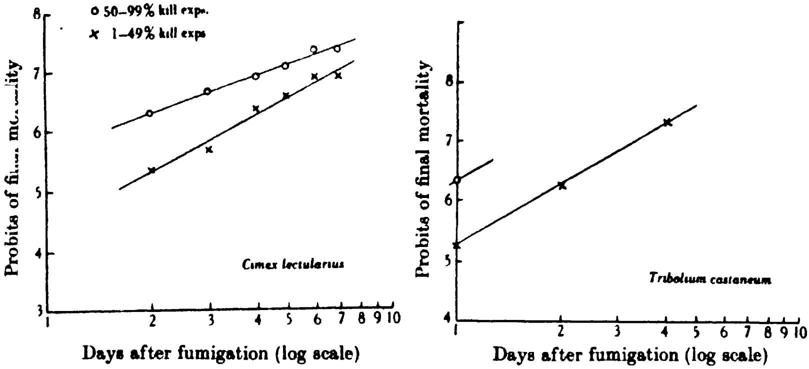

Fig. 2. Incidence of mortality on different days after fumigation.

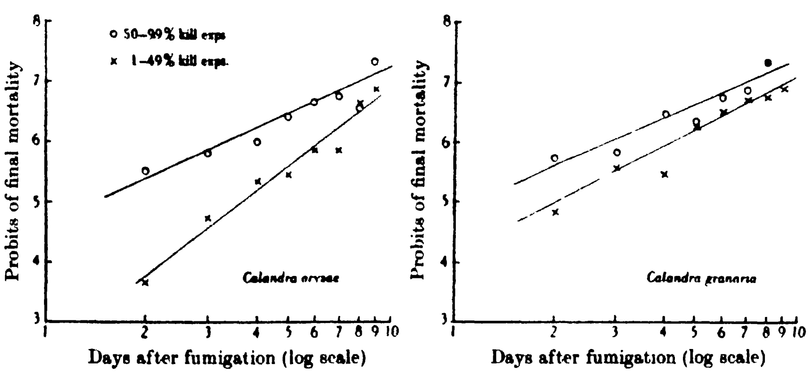

Fig. 3. Incidence of mortality on different days after fumigation.

A further interesting point emerges from the data if the mortalities given in Table IX are converted into probits to correct for random variation. If these values are plotted against the logarithm of the time after experiment it will be found (Figs. 2 and 3) that the relation is linear. This is in harmony with the observation of Bliss & Broadbent (1935), that recoveries of blow-flies stupefied with hydrogen cyanide give a symmetrical distribution, if the stupefaction time is measured in

logarithms. In both instances the time measured is that for a chain of biochemical processes. With the recovery from stupefaction it must deal with elimination of poison, while with ethylene oxide it probably depends upon conversion of the poison into an active form, possibly:

$$\text{Ethylene oxide} \xrightarrow[\textit{(hydrolysis)}]{} \text{ethylene glycol} \xrightarrow[\textit{(oxidation)}]{} \text{oxalic acid.}$$

Results

The results may be classified under two toxicity relationships. The data obtained at each exposure time give concentration—effect (i.e. percentage kill) regression lines and the whole series provide a time-concentration relation for a constant effect (percentage kill).

Both types of results can be regarded from various standpoints. In the first place it might be thought that the equations expressing inter-relations might contribute information about the fundamental processes of poisoning. Followers of Arrhenius have pointed out similarities between physiological equations and those expressing physico-chemical phenomena. For example the effect of temperature on toxicity is compared to its effect on the rate of chemical reaction (Reiner, 1933). Again the relation between concentration and time is compared with so-called "mono-molecular" reactions (Arrhenius, 1915). However, as Clark (1933) has pointed out, such similarities are probably superficial and misleading. The chief reason for this view lies in the extreme complexity of biological processes. If physicists hesitate to deal with the more complex forms of, for example, adsorption, it is apparent that physiology and toxicology are far beyond fundamental explanation at present. Apparently simple relations may be due either to a large number of contributing causes cancelling each other out, or else to some simple process (e.g. diffusion) being much slower than the others. In either case the relations of the whole chain of processes will be regulated by one, possibly simple, link.

The second reason for our inability to deduce much from toxicological results is the variability of biological material which makes it possible to fit several simple equations over the greater part of most curves.

It seems therefore that the chief value of relationships deduced from the data will be the empirical one of standardization of entomological fumigation results on the lines of analogous toxicological work. In spite of the extent of fumigation literature there have been very few attempts to do this. The main contributions are those of Strand (1930), Bliss (1934) and Bliss & Broadbent (1935). Until fumigation curves are expressed

in a simple general form it is impossible to find reliable criteria for resistance and toxicity which are naturally of considerable practical importance.

(1) *Concentration-mortality relationships*

It has been known for a considerable time that concentration-mortality curves are asymmetrically sigmoid in shape. As a result of this, it is not easy to use all the data in drawing the best possible curve,

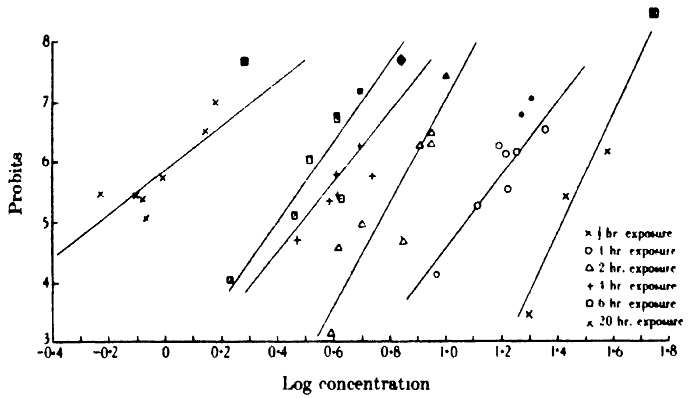

Fig. 4. Dose-kill regression lines for *Calandra oryzae*.

Table X

Calandra oryzae *results*

Exposure time hr.	Average no. of insects per experiment	Regression equation (x = log c; y = probits)	Degrees of freedom	χ^2	Probability $P>0{\cdot}05$* $P<0{\cdot}05$** $P<0{\cdot}01$***	50% kill concentration mg./l.
½	31·0	$y = 9{\cdot}4773x - 8{\cdot}4804$	1	4·1162	**	26·4400
1	25·2	$y = 7{\cdot}4328x - 3{\cdot}0445$	7	9·8117	*	12·0900
2	25·1	$y = 6{\cdot}8622x - 0{\cdot}2930$	6	23·8736	***	5·9060
4	27·1	$y = 6{\cdot}2214x - 1{\cdot}9184$	5	23·2401	***	3·1280
6	34·6	$y = 6{\cdot}5603x + 2{\cdot}3327$	5	20·6898	***	2·5510
20	27·7	$y = 3{\cdot}6207x + 5{\cdot}8322$	7	11·9826	*	0·5891

and the 99% kill position is, in particular, difficult to define. Accordingly, the 50% kill position (median lethal dose) was used as being the most reliable for comparative purposes (Tattersfield & Morris, 1924; Trevan, 1927; Strand, 1930).

A considerable contribution was made by Bliss (1934, 1935) who corrected the observed kills for random variation assuming a normal

distribution. He used as units "probits" equal to the normal standard deviation of mortality. To correct for the asymmetry still observed, he plotted the dose in logarithms. Whatever the fundamental causes, this gives a good fit for most similar data over the higher range of doses. At low doses discontinuity is sometimes observed. O'Kane *et al.* (1934) claimed to have eliminated this discontinuity by using *logarithms* of

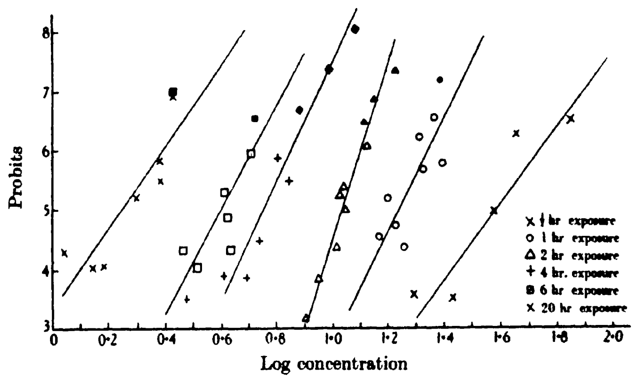

Fig. 5. Dose-kill regression lines for *Calandra granaria*.

Table XI

Calandra granaria *results*

Exposure time hr.	Average no. of insects per experiment	Regression equation ($x = \log c$; $y =$ probits)	Degrees of freedom	χ^2	Probability $P > 0{\cdot}05$* $P < 0{\cdot}05$** $P < 0{\cdot}01$***	50% kill concentration mg./l.
½	27·6	$y = 6{\cdot}6091x - 5{\cdot}5499$	3	3·6172	*	39·480
1	29·0	$y = 9{\cdot}4694x - 6{\cdot}7253$	8	30·8859	***	17·310
2	34·6	$y = 14{\cdot}2982x - 9{\cdot}8095$	8	13·0721	*	10·860
4	34·7	$y = 10{\cdot}0265x - 2{\cdot}5902$	7	21·2891	***	5·715
6	38·6	$y = 8{\cdot}5795x - 0{\cdot}1420$	5	27·5350	***	3·975
20	33·1	$y = 6{\cdot}8607x - 3{\cdot}3304$	7	29·4396	***	1·752

probits against logarithms of doses. Bliss (1935), however, comments that a logarithm of a probit is not a natural function. The same effect could have been obtained with an exponential equation. However, he claims that a logarithmic equation fits all but the lowest doses as well as exponential or hyperbolic functions and with one loss constant. Moreover, the logarithmic formula gives the highest 99% theoretical dose and hence, as a practical consideration, the greatest margin of safety.

Because of these advantages and because it was found to give a reasonably good fit with the data presented in this report, which mainly deals with the higher dose range, Bliss's method has been employed for the statistical analysis of results.

The results may be seen plotted graphically in Figs. 4–7, and the essential statistics of each set of data are set out in Tables X–XIII.

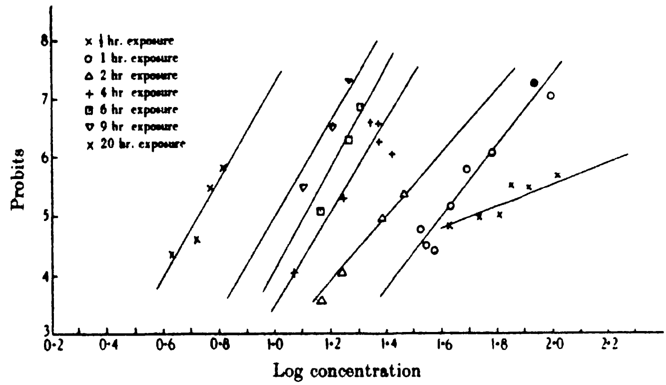

Fig. 6. Dose-kill regression lines for *Tribolium castaneum*.

Table XII

Tribolium castaneum *results*

Exposure time hr.	Average no. of insects per experiment	Regression equation ($x = \log c$; y = probits)	Degrees of freedom	χ^2	Probability $P > 0{\cdot}05$* $P < 0{\cdot}05$** $P < 0{\cdot}01$***	50% kill concentration mg./l.
½	31·5	$y = 1{\cdot}8876x + 1{\cdot}727$	4	3·5527	*	54·190
1	35·7	$y = 6{\cdot}1578x - 4{\cdot}909$	6	7·3399	*	40·660
2	25·5	$y = 5{\cdot}4921x - 2{\cdot}716$	2	0·0906	*	25·400
4	30·1	$y = 7{\cdot}7653x - 4{\cdot}281$	4	7·9298	*	15·680
6	29·7	$y = 8{\cdot}6603x - 4{\cdot}634$	1	0·1821	*	12·950
9	31·0	$y = 10{\cdot}5494x - 6{\cdot}196$	1	0·1173	*	11·520
20	34·0	$y = 8{\cdot}3650x - 1{\cdot}124$	2	3·5414	*	5·396

I am indebted to Dr Bartlett, statistician at Jealott's Hill Agricultural Station for the analysis of the results and for his comments.

The number of experiments at each exposure is equal to the number of degrees of freedom plus two. (One experiment being used up to fix the position and one the slope of the regression line.)

In the probability column, the level of P (indicated by asterisks) gives a measure of the uniformity of the insect populations.

Values below 0·05, found with each insect except *Tribolium*, indicate unexplained heterogeneity.

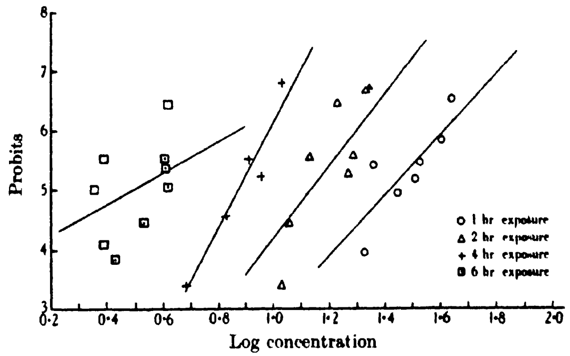

Fig. 7. Dose-kill regression lines for *Cimex lectularius*.

Table XIII

Cimex lectularius *results*

Exposure time hr.	Average no. of insects per experiment	Regression equation (x – log c; y = probits)	Degrees of freedom	χ^2	Probability $P > 0{\cdot}05$* $P < 0{\cdot}05$** $P < 0{\cdot}01$***	50% kill concentration mg./l.
1	21·6	$y = 5{\cdot}0743x - 2{\cdot}1828$	5	16·0273	***	26·03
2	24·5	$y = 7{\cdot}0854x - 3{\cdot}0401$	6	32·9310	***	13·63
4	28·2	$y = 8{\cdot}8641x - 2{\cdot}7361$	3	6·1033	*	7·40
6	24·8	$y = 2{\cdot}6475x + 3{\cdot}6934$	7	35·6051	***	3·12

(2) *Concentration-time relationships*

The relation between exposure time and concentration of fumigant to produce a constant mortality can be expressed by a simple hyperbolic equation as a first approximation:

$$c.t = W \text{ (Haber's formula).}$$

Two types of divergence from this equation have been claimed, one at low concentrations and one for short exposures, both resulting in increased values of W. It has already been stressed that certain common defects in technique produce similar results, and the divergences cannot be accepted as fundamental unless these errors have been eliminated.

A deviation at low concentrations beyond the limits of error has been observed by several workers. The $c.t$ curve may be said to flatten out. It is presumed that this is due to neutralization or excretion of the poison by tissues of the animal investigated. Assuming that the rate of elimina-

tion of poison is constant, the $c.t$ curve will approach asymptotically the highest tolerated concentration (c_0) instead of zero.

$$(c-c_0)\,t=W \text{ (Flury, 1921).}$$

This toleration effect, however, is by no means universal. Flury (1921) observed it with hydrogen cyanide but not with phosgene and suggested that the latter belongs to a class of gases which cannot be eliminated. Analogous results were found in the work on various poisons to fishes. Powers (1917) found theoretical threshold concentrations for most poisons to goldfish, but some metallic salts were exceptional. Carpenter (1927) showed that heavy metallic salts in general gave no indication of tolerated concentrations, and pointed out that their mode of action was peculiar. (They were not taken into the body, but acted by forming a film over the gills.)

The other deviation from the hyperbolic relation is noted in short exposures to high doses. This is treated in different ways by different authors. Hartmann (1918), working with *Cladocera* in solutions of inorganic salts, introduced a small time-toleration constant ("zeitlich Entgiftungsfaktor" $=t_0$) as well as a concentration tolerance factor. His formula, founded on a number of quite plausible assumptions, contains, however, no less than four constants. It is too cumbersome and not sufficiently well established for general application:

$$(c-c_0)\,(1-e^{-n(t-t_0)})=W,$$

$c_0=$threshold concentration, $t_0=$time tolerance factor, $n=$rate constant (depending on diffusion, etc.). The same type of deviation can be recognized in Powers's (1917) results where the slope of the "velocity of fatality" $\left(\frac{1}{\text{survival time}}\right)$ curve falls off above a certain point. Finally, Peters (1936), who regards the deviation as due to saturation of the tissues of the organism, proposes the correction

$$(c-c_s)\,t=W.$$

The value c_s is quite different from c_0; it is not a constant but represents the excess of concentration over the saturation of the tissues.

These short exposure deviations seem only to have been noted in experiments where the time measured was the survival of the organism in a toxic medium. It is, therefore, possible that the theoretical lag at infinite concentrations may be actually the time required for biological response to become manifest. If this is true, we should not expect the

deviation in the results of fumigation experiments since the time measured is merely exposure and not the period before death.

Support is given to this view by results of Peters (1936) (though he interprets them otherwise). He gives the results of some ethylene oxide experiments carried on until complete kills were obtained (Sofortwirkung) and others in which mortality was estimated 1–5 days after the exposures (Spätwirkung). The time-concentration products in the first series were found to be higher in the short than the long experiments, while in the second series no such difference was evident.

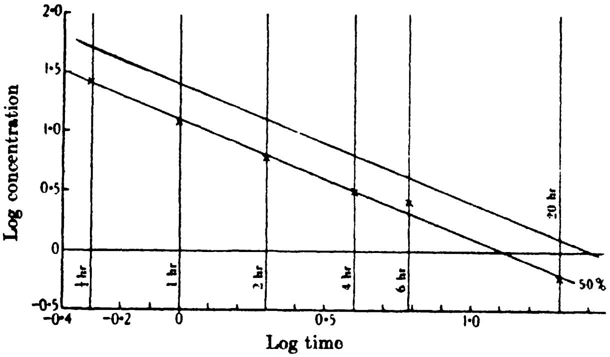

Fig. 8. Dose-time relations with *Calandra oryzae*.

Assuming that the present results, like the "Spätwirkung" series of Peters, do not show any short exposure deviations, they may be fitted with the simple empirical formula:

$$c^n t = W,$$

which gives a linear relation when converted to logarithms:

$$n \log c + \log t = K.$$

The slope of this line is given by n. Agreement with Haber's formula will be indicated by values about unity while the possibility of threshold concentrations are met by values of n greater than one.

To apply this formula to the present work, a constant mortality must be chosen. The 50% kill point has been taken because of reliability and general use. But since the regression lines do not show any constant change of slope, a similar result should be obtained with any other predetermined mortality. The figures have been treated statistically to determine if they will fit the equations within the limits to be expected from the individual regression lines.

The agreement between the observed results and the proposed formula can be judged from Figs. 8–11. Table XIV gives the equation constants for each insect and also statistical details.

A satisfactory fit was obtained for every insect except *Calandra granaria*. Here the high value of χ^2 suggests some inconsistency which is probably due to either the ½ hr. or 20 hr. point (see Fig. 9). The values

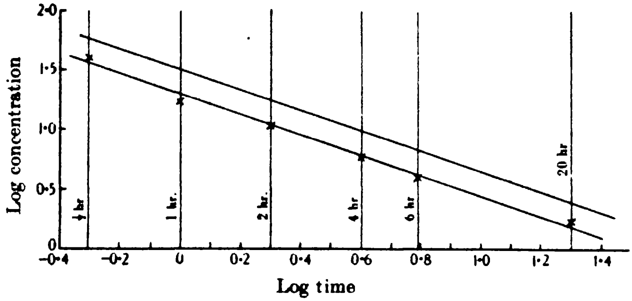

Fig. 9. Dose-time relations with *Calandra granaria*.

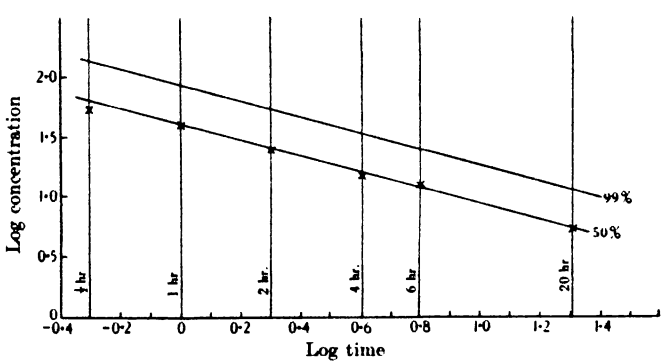

Fig. 10. Dose-time relations with *Tribolium castaneum*.

of P are not given since their precise significance is uncertain. They might reflect heterogeneity of material or measurement errors as well as departure from the proposed equations.

In general it is claimed that the formula $c^n t = W$ provides a satisfactory method of expressing concentration-time curves since no consistent deviations were shown by the four test insects.

A practical implication of the acceptance of this formula is the fact that only two exposure times need be investigated to solve the equation

and give the whole curve. This should economize the work of evaluating fumigants over a range of exposures.

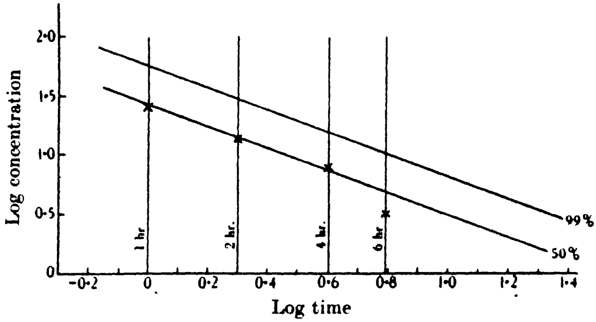

Fig. 11. Dose-time relations with *Cimex lectularius*.

Table XIV

Time-concentration relations

Insect	Regression equation $c^n t = W$	Standard error of n	Degrees of freedom	χ^2
Tribolium castaneum	$c^{1\cdot 81}t = 40\cdot 6$	0·033	5	11·149*
Calandra granaria	$c^{1\cdot 18}t = 19\cdot 3$	0·026	4	18·454
C. oryzae	$c^{1\cdot 00}t = 12\cdot 6$	0·031	4	5·882
Cimex lectularius	$c^{1\cdot 05}t = 26\cdot 8$	0·082	2	6·527

* Major fraction of this value due to one discrepancy which, when tested exactly, was found not to be significant. Hence no departure from calculated line for *Tribolium*.

(3) *Expression of toxicity and resistance*

(*a*) *Units.*

Examination of the literature concerning insect fumigants reveals a great lack of uniformity in expression of dose or concentration. Without considering the numerous ways of giving dosages there are four main ways of expressing concentrations attained. They may be mentioned as percentage by volume or by weight or as milligrams per litre, either under experimental conditions, or reduced to normal temperature and pressure. The percentages have the advantage that they denote a constant air-fumigant mixture, whatever the temperature, but the "milligram per litre" figures are easier to interpret into practical doses. As a compromise mg./litre at N.T.P. has been employed in this work, since it combines both the above advantages.

Time can be expressed conveniently in hours.

(*b*) *Criteria of resistance.*

Attempts to compare toxicities or resistances from published data are complicated by the variety of exposure times chosen. Usually only one period is chosen, and this may be anything from ½ to 48 hr. In order roughly to compare such heterogeneous data and to find a simple criterion of resistance, the general nature of time-concentration relations must be considered. In the previous section, we have found the equation $c^n t = W$, where $n = 1$ to 2 satisfactory. As an approximation the product $c.t$ may be taken as constant ($n \simeq 1$) and this provides a simple measure of resistance. It can be used to assess the effect of a practical fumigation where the concentration is variable. (If the course of the variation be plotted graphically the $c.t$ constant can be found by integrating the area under the curve: Peters & Ganter, 1935*a*.)

Unfortunately, the value of n is sometimes considerably greater than one (*Tribolium*—ethylene oxide = 1·5 (Table XIV); *Drosophila*—hydrogen cyanide = 1·8 (Bliss & Broadbent, 1935)). As a result the product $c.t$ increases steadily with longer exposures. Therefore, the value n must be mentioned in expressing resistance because some insects are relatively more resistant to long exposures than others.

Bearing in mind that the criterion should be brief and readily interpretable, it is proposed that it should consist of the 50 and 99% lethal concentrations for a 5 hr. exposure together with the value of n. From these figures the whole curves can be calculated if necessary. Table XV gives the relevant criteria for the four test insects to ethylene oxide at 25° C.

Table XV

Criteria of resistance to ethylene oxide

Insect	5 hr. 50% lethal concentration (mg./l. N.T.P.)	5 hr. 99% lethal concentration (mg./l. N.T.P.)	Value of n
Calandra oryzae	2·9	4·1	1·00 ± 0·031
C. granaria	5·5	8·4	1·18 ± 0·026
Cimex lectularius	6·6	12·3	1·05 ± 0·082
Tribolium castaneum	16·6	27·0	1·51 ± 0·033

SUMMARY

1. The orders of resistance of four different species of insects are shown to be different towards hydrogen cyanide, ethylene oxide and sulphur dioxide.

2. The different circumstances to be considered in fumigation research are surveyed with a view to ensuring reliable results. They can be grouped as:

(a) Factors which may influence absorption of poison.

(b) Factors which may influence toxic processes and degree of poisoning.

3. These factors are assessed and each aspect of technique is described with an estimate of the extent to which it has been controlled.

4. The arrangement of experiments to provide as much information as possible is described and the limits of accuracy defined.

5. Details of the rearing and treatment of insects are given.

6. Difficulties of estimation of mortality are considered and a relation between severity of fumigation and incidence of death or recovery from stupefaction is demonstrated.

7. The dose-kill data are treated by Bliss's Method of Probits and tables of statistics for each set of data are appended.

8. Various time-concentration relations are considered, and from these the empirical formula $c^n t = W$ has been adopted. A statistical analysis shows no serious deviation from this relationship.

9. The need for uniformity in expression of toxicity is stressed, and the following criterion of resistance proposed: "The 5-hr. concentrations (mg./l. N.T.P.) for 50 and 99% kill and the value of n".

10. The values of the criteria for the four species used (*Calandra granaria*, *C. oryzae*, *Tribolium castaneum* and *Cimex lectularius*) are given.

I should like to express here my deep gratitude to Prof. Munro for encouraging me to undertake this work and also to Dr Bovingdon, Dr Lubatti and Dr Bartlett for their technical advice.

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STUDIES ON AMERICAN FOUL BROOD OF BEES

II. THE GERMINATION OF THE ENDOSPORES OF *BACILLUS LARVAE* IN MEDIA CONTAINING EMBRYONIC TISSUES

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WITH AN APPENDIX

EXPECTED ERRORS IN DILUTING BACTERIAL SUSPENSIONS

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DURING the past few years the use of the tissues of the developing chicken embryo as a bacteriologically sterile culture medium for many animal and human viruses has greatly increased. Two general techniques have been developed, one involving the direct inoculation of the chorioallantoic membrane of the developing embryo (Burnet, 1936), and the other the preparation of an embryo "brei" by mincing whole embryos under aseptic conditions (Dochez *et al.* 1936; Li & Rivers, 1930; Magill & Francis, 1936; Nelson, 1936).

Bacillus larvae, the organism responsible for American foul brood, requires rather complex media for the germination of its endospores and multiplication of the vegetative cells arising therefrom (Lochhead, 1928; Maassen, 1919; Sturtevant, 1924, 1932; Tarr, 1937; White, 1907, 1920). This is hardly surprising since many of the bacteria pathogenic for man and animals are likewise fastidious with respect to their nutrient requirements, a large inoculum of such organisms frequently being necessary to initiate growth even on "rich" media (Knight, 1936). Sturtevant (1932) showed that, normally, several million spores of *B. larvae* are required to produce vegetative growth on a complex egg yolk carrot extract medium, and that the presence of reducing sugar (glucose) in this medium in concentrations slightly in excess of 2·5% either markedly inhibited, or entirely suppressed, the germination of the endospores and the multiplication of the vegetative cells of this species. It

occurred to the writer that the tissues of the developing chicken embryo might provide a nutrient substrate for *B. larvae* more favourable than those hitherto employed, and that, if this proved to be the case, the sensitivity of this organism to reducing sugars in various concentrations could be tested conveniently in this medium. The results of experiments designed to test these points are recorded below.

Experimental

The spore suspensions of *B. larvae* employed were prepared directly from the ropy remains of larvae dead of American foul brood under aseptic conditions, and not from pure cultures of this organism as in previous experiments (Tarr, 1937). The ropy larvae, which contained *B. larvae* spores in apparently pure culture, were ground in distilled water, the coarse debris being removed from the resulting suspension by passing it through two layers of Whatman No. 1 filter paper. The spores in the filtrate were washed by centrifuging and were suspended in water. The suspensions were stored at about 2° C. and were used within 10 days of preparation. The number of spores per ml. in such suspensions was estimated by means of a Thoma haemocytometer slide, a large number of microscopic fields being counted in each instance. The standard error for each total count was kindly estimated by Mr Cochran. Serial dilutions were prepared from these suspensions, immediately prior to use, by mixing the suspension thoroughly, removing 1 ml. with a sterile pipette (accurate within approximately $\pm 2\%$), adding this volume to 9 ml. of sterile distilled water, and repeating the procedure until the desired set of dilutions was obtained. This dilution method is subject to certain inaccuracies which are discussed in an appendix to this paper (see p. 640). In the experiments to be described, in stating the number of spores introduced with each inoculum due allowance has been made for both the error due to the method of counting and to that of making the dilutions.[1] There is the possibility that a certain proportion of the *B. larvae* spores in such suspensions are "non-viable" even under ideal conditions but, unfortunately, there is no reliable method of determining whether such spores are present.

The egg yolk and beef digest brood filtrate agar media were prepared as previously described (Tarr, 1936). Embryo brei medium was prepared usually from 14-day-old chicken embryos, but occasionally from 12-day-old embryos, by mincing them in Tyrode solution (pH approximately 7·2), following, in general, the method of Li & Rivers (1930). 20–25 ml.

[1] I am indebted to Mr Cochran for determining these errors.

of Tyrode solution was used for each embryo. Immediately after preparation portions of the brei were pipetted into sterile test tubes, using wide mouth glass pipettes. There is always danger that a medium prepared in this manner may be contaminated, and, unfortunately, it cannot be tested for sterility by the customary method of incubating prior to inoculation because the developing embryonic tissues die fairly rapidly, especially at higher temperatures of incubation. However, experience has shown that if the medium is prepared as quickly as possible, with all reasonable aseptic precautions, it is rarely contaminated. It has usually proved easy to differentiate *B. larvae* from the contaminating organism, and to isolate this bacillus in pure culture. In a few instances *B. larvae* was cultivated on the chorioallantoic membrane of the developing embryo, the technique described by Burnet (1936) being followed.

The various media were inoculated with aqueous suspensions of *B. larvae* spores and incubated at approximately 35° C. When a microscopical examination of a given culture, made by streaking a portion of the medium on a slide with a small amount of a saturated aqueous solution of nigrosine, indicated positive growth of *B. larvae*, the presence of this organism in pure culture was confirmed by the following tests. Transfers were made on to beef digest brood filtrate agar slopes, and the characteristic cultural and morphological features of the resulting cultures were recorded. All cultures were tested for nitrite since Lochhead (1928, 1937) has shown this test is strongly positive when *B. larvae* has grown in many culture media even without added nitrate. In the writer's experiments it was found that nitrite was not produced when *B. larvae* grew in embryo brei medium unless nitrate was added to the medium. Thus, a positive nitrite test resulted when one or two drops of a sterile 5% solution of potassium nitrate were added to an embryo brei culture in which *B. larvae* was growing and the medium was reincubated for 16–24 hr. at 35° C.

Exp. 1. A suspension containing $141 \times 10^6 \pm 2{\cdot}8\%$ spores of *B. larvae* per ml. was used. Embryo brei was tubed in 5 ml. portions in $6 \times \frac{5}{8}$ in. test-tubes, and egg-yolk and beef digest brood filtrate agar were sloped in 6 ml. amounts in similar tubes. These media were inoculated with 1 ml. portions of the various dilutions of the above spore suspensions, the agar cultures being sealed with paraffin wax in order to prevent undue drying of the media on prolonged incubation. The chorioallantoic membranes of 10–12-day-old chicken embryos were inoculated with 0·01 ml. of spore suspensions using an Agla micrometer syringe, a slight rupture being made in the membrane if this had not occurred on separation of shell and chorioallantoic membranes. All cultures were incubated at 35° C., periodical microscopical examinations being made

of the embryo brei and chorioallantoic membrane cultures to ascertain whether or not growth had occurred. A maximum period of 30 days was given for incubations. Although it is possible that spores may remain dormant for longer periods this length of time was considered adequate for the experiments recorded. The results of this experiment are given in Table I, the number of tubes of medium inoculated from each dilution being recorded in brackets beside the number of tubes showing positive growth within the 30-day period.

Table I

The ability of B. larvae *spores to germinate in different media*

No. of spores of *B. larvae* introduced (standard error due to counting and dilution factors given)	No. of cultures of those inoculated showing growth of *B. larvae* (no. of tubes inoculated in brackets): Egg-yolk agar	Beef digest brood filtrate agar	Embryo brei	Chorioallantoic membrane
$141 \times 10^6 \pm 2{\cdot}8\%$	(6) 3	(6) 5	(2) 2	—
$141 \times 10^5 \pm 3{\cdot}0\%$	(6) 0	(6) 3	(2) 2	—
$141 \times 10^4 \pm 3{\cdot}1\%$	(6) 0	(6) 0	(2) 2	(1) 1
$141 \times 10^3 \pm 3{\cdot}3\%$	(6) 0	(6) 0	(2) 2	(1) 1
$141 \times 10^2 \pm 3{\cdot}5\%$	(6) 0	(6) 0	(2) 2*	(1) 1
$141 \times 10^1 \pm 4{\cdot}4\%$	(6) 0	(6) 0	(2) 2	(1) 1
$141 \pm 9{\cdot}2\%$	(6) 0	(6) 0	(5) 4	(1) 1
$14 \pm 26{\cdot}9\%$	—	—	(3) 0	—
1·4	—	—	(3) 0†	—
0	—	—	(3) 0	—

* One contaminated by a coccus but *B. larvae* isolated in pure culture.
† One contaminated by a torula; no sign of *B. larvae*.

It is evident from these results that both the embryo brei medium and chorioallantoic membrane are much more favourable substrates for the germination of *B. larvae* spores than either of the agar media. Only the limited number of experiments recorded in Table I have been made with the chorioallantoic membrane technique, and it would be interesting to make a large number of inoculations using embryos of different ages and various doses of spores. Further experiments recorded below have all been made using embryo brei medium.

Exp. 2. A suspension containing $105 \times 10^6 \pm 3{\cdot}2\%$ *B. larvae* spores per ml. was used. Dilutions were prepared from this suspension in the usual manner and 1 ml. portions were inoculated into tubes containing 5 ml. of embryo brei medium each. Two different series of experiments were made using various numbers of tubes of embryo brei prepared from different embryos and various doses of spores. The results of this experiment are set out in Table II.

From the limited data so far obtained it seems inadvisable to attempt to make any prediction regarding the number of *B. larvae* spores which can be expected to germinate on embryo medium. It would be interesting to make a large series of inoculations using different batches of embryo

brei and inocula of spores ranging from approximately 10,000 to ten from various spore suspensions. It appears, from the relatively small number of experiments made, that 1000 spores will usually cause growth while ten spores are extremely unlikely to do so.

Table II

Germination of B. larvae *spores in embryo brei medium*

No. of spores of *B. larvae* introduced (standard error due to counting and dilution factors given)	No. of tubes inoculated	No. of tubes showing growth of *B. larvae*
	A	
10,500 ± 3·9%	3	3*
1,050 ± 5·0%	3	3
105 ±10·6%	3	1
10·5 ±31·1%	3	0†
1	3	0
	B	
1,050 ± 5·0%	8	7
105 ± 10·6%	8	3
10·5 ± 31·1%	8	0‡

* One contaminated but *B. larvae* isolated in pure culture.
† One contaminated by a coccus, no sign of *B. larvae*.
‡ One contaminated by an aerobic bacillus, no sign of *B. larvae*.

In further experiments the effect of added available nitrogen, in the form of beef digest broth, and reducing sugars on the germination of *B. larvae* spores in embryo brei have been investigated.

Exp. 3. Tubes containing 4 ml. of embryo brei and 1 ml. of Hartley's beef digest broth were prepared, and these were inoculated with various dilutions of the spore suspension used in Exp. 2. The results obtained are given in Table III.

Table III

The effect of beef digest broth on germination of B. larvae *spores in embryo brei medium*

No. of spores of *B. larvae* introduced (standard error due to counting and dilution factors given)	No. of tubes showing positive growth of *B. larvae* out of four inoculated
105×10^4 ±3·5%	4
105×10^3 ±3·6%	4
105×10^2 ±3·9%	4
105×10^1 ±5·0%	3
105 ±10·6%	0*
10·5 ±31·1%	0

* One contaminated by a coccus.

From the results obtained it appears as if beef digest broth hinders rather than promotes germination of *B. larvae* spores in embryo brei medium.

Exp. 4. Embryo brei medium was prepared with added beef digest broth (this was added before it was found that added nitrogen appears to hinder slightly the germination of *B. larvae* spores in the embryo medium). Various amounts of 25 or 50% aqueous solutions of an equimolecular mixture of fructose and glucose (sterilized by autoclaving) were added to the brei medium so that concentrations of reducing sugar in the final media varied from 0 to 12·5%. Each tube was inoculated with 0·1 ml. of a dilution of the spore suspension used in Exp. 2, $105 \times 10^3 \pm 3{\cdot}6\%$ spores being introduced. In Table IV the set up and results of this experiment are given. No apparent deleterious effect of even 12·5% reducing sugar on germination of *B. larvae* spores was observed. In all cases positive growth of *B. larvae* took place within 3 days of inoculation, and in most instances within 1 or 2 days. This experiment is open to criticism on the grounds that the embryonic tissues might destroy the reducing sugar, thus rendering the medium suitable for the growth of *B. larvae*; while this point has not been checked by measuring the amount of reducing sugar present in the media after *B. larvae* has commenced to grow, it is extremely doubtful if the amount of tissue present would destroy more than a fraction of a 12·5% solution of reducing sugar. The *p*H of the media after the growth of *B. larvae* had occurred was about 4·5; and those containing the higher concentrations of reducing sugars were still distinctly sweet to the taste.

Table IV

Germination of the endospores of B. larvae *in embryo brei medium containing reducing sugars*

Composition of medium: Embryo brei ml.	Glucose-fructose solution ml.		Beef digest broth ml.	Spore suspension ml.	H_2O ml.	Final concentration of reducing sugar %	No. of cultures showing growth (quadruplicates)
3	0	25%	0·9	0·1	1·0	0	4
3	0·2		0·9	0·1	0·8	1	4
3	0·4		0·9	0·1	0·6	2	4
3	0·6		0·9	0·1	0·4	3	4
3	0·8		0·9	0·1	0·2	4	4
3	1·0		0·9	0·1	0·0	5	4
2·5	0·5	50%	0·4	0·1	0·5	6·25	4
2·5	0·75		0·4	0·1	0·25	7·38	4
2·5	1·0		0·4	0·1	0·0	12·5	4

Discussion

Embryo brei prepared from chicken embryos, and the chorioallantoic membrane of the developing egg, are by far the most favourable media yet found for the germination of the endospores of *Bacillus larvae* and, in these substrates, the vegetative cells of this organism multiply rapidly. Added available nitrogen in the form of beef digest broth to the embryo brei medium tends to inhibit rather than favour the germination of the spores.

It is of interest that germination of the spores and multiplication of the vegetative cells of *B. larvae* took place in the presence of concentrations of reducing sugars as high as 12·5% in embryo brei medium. Higher concentrations have not yet been tried. This finding is rather unexpected in view of the fact that Sturtevant (1924) found that relatively low concentrations of glucose (2–3%) either seriously impaired, or totally inhibited, spore germination or vegetative cell multiplication of *B. larvae* on egg-yolk agar medium. He suggested that the reason that *B. larvae* normally attacks larvae only after the cells containing them have been sealed is because, at this stage, the reducing sugar in them has fallen to such a low level that it ceases to inhibit the spores of this organism from germinating. This attempted correlation of the events occurring in a culture medium with those taking place in the larva itself is open to the criticism that the factors which tend to inhibit growth of *B. larvae* in a culture medium are totally different from those operating in a living organism. At present, it cannot be stated definitely that reducing sugar concentration plays any role in determining at what stage in larval life American foul brood develops. Much remains to be learned regarding the "age incidence" of American foul brood, especially in view of the body of evidence which has recently been accumulated (Tarr, 1937, 1938) relating to the probable part played by the adult bee in carrying the disease.

Summary

The tissues of the developing chicken embryo form a more suitable substrate for the germination of the endospores of *Bacillus larvae* than any medium so far described.

The addition of beef digest broth to a medium of minced chicken embryo hinders rather than promotes the germination of *B. larvae* spores.

Concentrations of reducing sugars as high as 12·5% cause no apparent hinderance in the germination of *B. larvae* spores in embryo medium.

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APPENDIX

EXPECTED ERRORS IN DILUTING BACTERIAL SUSPENSIONS

BY W. G. COCHRAN

From a solution containing a known number of spores or vegetative cells per unit volume, higher dilutions are commonly prepared by extracting a small measured volume in a pipette (usually 1 ml.) and adding this to a larger volume of water (usually 9 or 99 ml.). It is realized that if a number of, e.g. $\frac{1}{100}$, dilutions are prepared from the same solution, these dilutions will not all contain exactly $\frac{1}{100}$ of the original number of, e.g. spores, per unit volume. With skilful work, the two principal sources of variation appear to be (1) the sampling variation involved in taking out a small measured volume by pipette; (2) errors in the volume actually removed by pipette. A 1 ml. pipette will not always remove exactly 1 ml. and, indeed, most manufacturers specify the limits of error of their pipettes.

Assuming that the solution is thoroughly mixed at each stage of the dilution before removing a small volume by pipette, and that there is no clumping of the spores or vegetative cells on mixing, the sampling

errors in the number of spores removed per unit volume will follow a Poisson series distribution and are known if the mean number of spores per unit of the original volume is known. An allowance may also be made for the measuring errors of the pipette, according to its standard of accuracy. This allowance may also be made to include errors in the measurement of the volume of water to which the solution in the pipette is added on making the dilution, though it seems likely that these errors will be relatively negligible. Thus, from theoretical considerations alone, we can assign limits of accuracy to the number of spores or bacterial cells in the volume of solution which is being used in any dilution experiment. These limits are to be regarded as attainable with competent work.

Table V

Variation due to dilution and pipetting

No. of spores etc.	Standard error %	5% limits Lower %	Upper %	No. of spores etc.	Standard error %	5% limits Lower %	Upp %
10^6	1·00	98·0	102·0	120	9·18	82·8	118·8
10^5	1·05	97·9	102·1	100	10·1	80·8	120·8
2×10^4	1·22	97·6	102·4	90	10·6	79·8	121·7
10^4	1·41	97·2	102·8	80	11·2	78·6	123·1
4×10^3	1·87	96·3	103·7	70	12·0	77·2	124·8
2×10^3	2·45	95·1	104·9	60	13·0	75·4	126·9
$1{\cdot}4\times10^3$	2·85	94·3	105·7	50	14·2	73	130
10^3	3·32	93·4	106·6	45	14·9	72	131
800	3·67	92·9	107·3	40	15·8	70	133
600	4·20	91·9	108·4	35	16·9	68	136
500	4·58	91·2	109·2	30	18·3	65	139
400	5·10	90·2	110·3	25	20·0	62	143
300	5·86	88·8	111·8	20	22·4	57	149
200	7·14	86·6	114·4	15	25·8	49	157
150	8·23	84·5	116·7	10	31·6	38	172

Note. The standard error due to pipetting was assumed to be 1% in computing the above table.

Table V shows, for a wide range of concentrations, the standard error percentage of the number of spores or vegetative cells and the lower and upper limits (also as percentages) within which the number will lie in 95% of cases. It must be stressed that these errors refer to the number of spores in that volume of solution which is being used for experimental purposes. To take an example, suppose that a solution containing $1{\cdot}5\times10^5$ spores per ml. is diluted to $\frac{1}{1000}$ and that 1 ml. of the dilute solution is removed for experimental purposes, e.g. the inoculation of an experimental medium to ascertain the number of spores which will initiate growth. The expected number of spores in 1 ml. of the dilute solution is 150. Reference to the diagram shows that the

standard error of this figure is 8·2 % and that the 95 % lower and upper limits are 84·5 and 116·7 % respectively. Thus the number of spores in the ml. of solution is fairly certain to lie between 84·5 and 114·4 % of 150, i.e. between 127 and 175. If, however, only $\frac{1}{10}$ ml. of this dilution is being used, the reading must be taken at the point 15 and not at 150, since there are only about 15 spores in the volume which is actually being used. The standard error at this point is 25·8 % and the limits of accuracy are 7·35 and 23·55, or, taking the widest *integral* range within these limits, between 8 and 23.

With numbers of spores below 50, it is not possible to give 5 % limits which are at all exact, since the number of spores must be a whole number with a finite probability. For instance, the exact probability of getting numbers of spores outside the limits 8 and 23 in the above example is 3·7 % instead of 5 %, while the probability of getting numbers outside the limits 7 and 22 is 7 %.

The entries in Table V are not evenly spaced, as the standard error and limits change more rapidly as the dilutions become higher. The range is, however, sufficiently well covered to give reasonably accurate limits for values intermediate between those shown in Table V. Proportional parts will not, however, give a good interpolation in the lower part of the table. If accurate interpolation is required, proportional parts should be used on the reciprocals of the square roots of the numbers of spores.

To construct Table V, some assumption was necessary about the error of pipetting, though this is important only at low dilutions. The standard error was assumed to be 1 %. Thus, in all but 5 % of cases, the volume extracted by the pipette was assumed correct to within 2 %. This probably represents the limits of error of good pipettes. The table can be constructed for any given standard of accuracy in pipetting; however, to find the limits of variation in the number of spores with pipetting which is correct to say, 4 %, it is sufficiently accurate for most purposes to regard the 5 % limits of accuracy as ± 4 % until the dilution is sufficiently high that the limits in the table rise above 4 % and, thereafter, to use the limits in the table. In this connexion it should be noted that after, e.g. three dilutions using pipettes with a 1 % standard error, the standard error of the dilute solution due to pipetting is, not 1 %, but $\sqrt{3}\,\% = 1{\cdot}73\,\%$; and if the dilution is still so low that sampling errors are negligible, the 5 % limits of accuracy are ± 3·5 %.

Table V covers the range from 10 to 10^6 units. Below 10 units a single sample is extremely variable. Above 10^6 units the whole error is due to pipetting.

The number in the original suspension will not usually be known exactly, but will itself be estimated with a known standard error. In this case the standard error of a dilute suspension is found by adding the square of the standard error in Table V to the square of the original standard error, and taking the square root. The standard errors given in Tables I, II and III were obtained in this way. As an example, we will derive the standard error of the dilution 141×10^1 in Table I. The original standard error is 2·8%, and from the table the standard error due to dilution, with a 1% pipette standard error, is also about 2·8%. Since, however, there have been five successive dilutions, the pipetting standard error is $\sqrt{5}$%. Thus the standard of the dilute suspension is

$$\sqrt{\{(2{\cdot}8)^2 + (2{\cdot}8)^2 + (5-1)\}} = 4{\cdot}4\,\%.$$

If the additional error due to repeated pipetting were ignored in the above calculation, as previously suggested, the result would be 4·0%.

(*Received* 4 *January* 1938)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

ORDINARY MEETING of the Association held at 2.30 p.m. on Friday, 18 March 1938, at the Imperial College of Science and Technology, London, the President, Mr C. T. GIMINGHAM, in the Chair.

Discussion on the Use of Chemical Weedkillers

The following papers were read:

I. Chemical weedkillers in relation to horticulture. By M. A. H. TINCKER, M.A., D.Sc.

II. Some factors influencing the agricultural use of chemical weed-killers. By R. K. MACDOWALL, Dipl.R.T.C., A.M.I.Chem.E.

III. The relative toxicity of chemical weedkillers. By G. E. BLACKMAN, M.A.

IV. The control of weeds in lawns and fine turf. By R. B. DAWSON, M.Sc., F.L.S.

V. Chlorate weedkillers. By O. OWEN, M.Sc., Ph.D., A.I.C.

I. CHEMICAL WEEDKILLERS IN RELATION TO HORTICULTURE

BY M. A. H. TINCKER, M.A., D.Sc.

Keeper of the Laboratory, Royal Horticultural Society, Wisley, Ripley, Surrey

IN 1934 the experimental work carried out at Wisley was reported, together with a brief review of the literature. In the ensuing interval further experience has been gained, chiefly from advisory work, of the relative success or failure of several chemical weedkillers when used in frequent conjunction with the usual routine carried out in different departments of the industry. It will be convenient to consider these departmental aspects separately.

Orchards

(*a*) *Cultivated.* Only with the greatest care can sodium chlorate be used to eradicate the arable weeds in cultivated orchards. Nettles (*Urtica dioica* and *U. urens*), groundsel (*Senecio vulgaris*) and annual meadowgrass (*Poa annua*), fat-hen (*Chenopodium album*), chickweeds (*Cerastium vulgatum* and *Stellaria media*), chamomiles (*Anthemis arvensis* and *A. Cotula*), and other weeds can be killed quickly in early autumn by a 2½–3% spray of sodium chlorate. This is a most inconvenient time, as the fruit harvest is then in progress. In early spring smaller weeds can be eradicated similarly. Approximately 1 gal. of solution to 10 sq. yd. suffices for a

fairly thick covering of weeds; for smaller weeds a lighter application may suffice. The spray solutions must be applied carefully and the ground must not be soaked, especially where surface rooting of the fruit trees has been encouraged by previous manurial treatments. Where smaller fruit has been interplanted between standard top fruits further care in spraying is necessary. The tar oil washes used in early spring check the growth of many common weeds, but recovery usually takes place later.

Cyanamide is useful on soils with a low nitrogen content. It can be used for several consecutive seasons; by application to the weeds when they are wet and by the subsequent bruising and burial by mechanical or other cultivation processes, accelerated decomposition to humus is facilitated and the tilth and soil composition is thus improved. Cyanamide fails to eradicate couch grass (*Agropyron repens*) without repeated cultivation.

Arsenical weedkillers are unsuitable for continued orchard use; their toxicity is low and the arsenic tends to remain in the soil, though Crofts (1933) found no serious soil damage from acid arsenical sprays.

There appears to be little or no readily available information concerning the use of sulphuric acid in fruit orchards. Only a small amount of damage to the soil, due to decomposition of the carbonates, results from application of sulphuric acid used to kill annual weeds under arable field conditions. The method is worthy of further tests.

(*b*) *Grass orchards*. Where there is a grass covering it is somewhat safer to use sodium chlorate as a weedkiller to eradicate in this case nettles (*Urtica* spp.), ragwort (*Senecio Jacobaea*), and thistles (*Cirsium acaule*, *C. arvense*, and *C. lanceolatum*). Every care must be taken to apply the spray of the solution of the weedkiller to the weeds only and to prevent drenching or soaking the ground. If only light applications be given carefully to the fully developed foliage of the weeds the method is successful and no damage results to the trees.

It is seen that weed eradication in orchards cannot be accomplished by chemical weedkillers without much care.

Market gardens and vegetable growing

The poisonous nature of the arsenical weedkillers, and the likelihood of food contamination, generally precludes their use. All the vegetables tested, as yet, are very sensitive to damage from chlorates; so that during the growing periods and for some time previously, determined by the soil conditions as they influence drainage and percolation, the application of chlorates as weedkillers is not practicable. Korsmo (1932) tested numerous vegetables to ascertain their reaction to 4% solutions of sulphuric acid. He reported the following results:

(*a*) Undamaged by the acid: cabbage, cauliflower, leek, onion, and shallot.

(*b*) Damaged but recovered: peas, lettuce, swede, salsify, purslane, dill, thyme.

(*c*) Damaged as severely as the weeds: carrot, radish, beetroot, celery, parsnip, broad bean, dwarf bean, French bean, parsley.

Thus, the use of sulphuric acid is very restricted in market garden work.

There can be no question of the value of cyanamide in the previous preparation of weedy ground for vegetable culture, since the improved quality of vegetables when

grown with adequate humus in the soil is undoubted; this is additional to the manurial value of the mineral constituents of the fertilizer.

It appears, then, that cultivation methods must still be relied upon for weed eradication in this section.

Lawns and grass

The Bingley methods, advocated by Dawson & Evans (1931 *a*), of eradicating weeds by a mixture of ammonium sulphate and ferrous sulphate have, in general, given satisfactory results on soils of the Bagshot sands, Folkestone beds, London clays and other non-calcareous soils. For ecological details of closely cut turf so treated Blackman's paper (1932*a*) should be consulted. But the eradication of weeds from lawns on chalk soils, such as the downlands, is more difficult. To build up a lime-free surface layer is often expensive, and the repeated application of sulphate of ammonia and iron, followed by repeated cuttings of the grass, frequently results in thin poor grass if little or no other fertilizers be given for several seasons. Moreover, the weeds do not so readily disappear on these soils by such methods. The "acid theory" is inapplicable where the surface layers contain much chalk, but a chalk-free layer is not unknown above pure chalk.

How does *Brachypodium pinnatum* compete against the chalkland weeds? Perhaps an ecological study might reveal interesting data although this species is not itself likely to be useful.

In America the use of weak solutions of chlorate to eradicate such weeds as *Glechoma hederacea* has been recommended by Walton (1929). At 2½%, grass may be severely checked, as in our tests when discoloration was apparent, but ultimate recovery took place. This method may be of value in conjunction with later resowing of the grass. A solution of 1% sodium chlorate will injure certain weeds of lawns and cause less damage to the grass, but the method has few real advantages to offer in comparison with a "spot" treatment with sulphates of ammonia and iron.

Hard tennis courts

Hard tennis courts are sometimes laid down on soils from which the root systems of weeds have not been adequately removed. Bindweed (*Convolvulus arvensis*) may spread very rapidly in the moist coarse ashes frequently used below the granite chips. In one case thousands of shoots appeared through the granite type of surface within a few days. By permitting the early stages of leaf development the use of a 5% solution of sodium chlorate proved highly effective when applied at 2 gal. for each 10 sq. yd., and no subsequent trouble with this weed was reported. Subsequent watering was carried out to wash the remaining chemical into the lower layers of the court. Unless this is done the granite chips dry at a rate slower than usual, and the danger of fire remains. (Calcium chlorate decreases the rate of drying.)

Paths and drives

Generally, the application of sodium chlorate at 5% (or if no grass is present, at 2½%) is effective for one season. Arsenical weedkillers, such as 2% sodium arsenite, often have a more persistent effect, lasting for 2 or 3 years at a similar rate of application. It has been observed fairly frequently, in damp localities, that subsequent invasion by mosses takes place readily some months after chlorate has been used.

There is a fire danger arising from use of chlorates on drives where flints may cause a spark as motor cars pass but, fortunately, no cases have been reported of serious damage in this country.

Neglected ground

Sulphuric acid at 10% has sometimes failed to kill large tufts of certain grasses including *Dactylis glomerata*, *Holcus lanatus*, and *Alopecurus pratensis*, but has generally proved effective when applied to *small* broad-leaved weeds.

If there are *many* thick tussocks of well-established grass it has been necessary to use a 10% solution of sodium chlorate to effect complete killing at 1 gal. to 10 sq. yd. Vegetation so killed may be raked together and burnt, but labour is required to clear the debris. The fallow period for soil recovery varies inversely as the rate of the drainage; a short period of two months was sufficient on certain Bagshot sands in winter to permit of sowing lupins and lettuce. On heavier soils, five or six winter months should elapse before any seed sowing or planting can be safely undertaken.

Mention has already been made of the value of cyanamide in conjunction with cultural methods and smother crops for eradicating couch grass and the subsequent rotting of the vegetable matter. The tests made by Scarlett (1937) at Musselburgh and elsewhere, show that a period of 12–15 months is required for this process; it appears unlikely that this period can be materially reduced advantageously.

Acid arsenical weedkillers, which combine the application of sulphuric acid and arsenite, have been reported by Crofts (1933) to give satisfactory results in the eradication under Californian conditions, of certain very troublesome weeds, such as *Convolvulus arvensis* and *Centaurea repens*. For the reaction to sulphuric acid alone, the lists of species drawn up by Aslander (1927) should be consulted, where it will be seen that the leaves with narrow foliage frequently survive an application that kills broad leaved weeds.

Miscellaneous eradication

For eradication of trees, ivy on walls or trees, and many climbers, arsenical pastes containing sodium arsenite are effective. The usual method of application is to drill holes in the wood, fill with the arsenite, and cover or plug with clay.

Sodium chlorate may be applied to gorse as a dry powder, but it is necessary to clean thoroughly any mechanical spreader or duster before and after use. The application of the dry powder is useful in killing other plants also, but an even spread is not so readily obtained as by means of solutions.

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II. SOME FACTORS INFLUENCING THE AGRICULTURAL USE OF CHEMICAL WEEDKILLERS

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I propose to consider first some of the snags in the use of weedkillers by farmers, for with the wealth of chemicals available and the prevalence of weeds, it seems strange that less than 1% of the land under corn in this country was sprayed last year. Although the sellers of most of the chemicals stress, and rightly so, the reasonable cost per acre, of, shall we say, x shillings, this cost can be attained only after a capital expenditure of ys. for a machine. To put things algebraically, y is generally more than 100 times x at the current prices of modern spraying equipment. For this unfortunate state of affairs I am afraid the engineer is to blame, since he has failed to build a machine that is both cheap and durable for the most effective and popular corrosive sprays such as sulphuric acid. Until the price of acid-resistant alloys falls, or that of corn rises, it is difficult to avoid a heavy charge per acre for depreciation. The manufacturers of weed sprays are fully alive to the importance of this problem and have done much to encourage the production of satisfactory machines. They have even proposed the organization of a technical trial with a substantial money prize for the best machine submitted in open competition.

I imagine that some of you are wondering why the farmer is so reluctant to disburse the cost of a machine when most of the published results of randomized plot experiments with sulphuric acid in this country show increased yields of 8–10 cwt./acre

of corn. In terms of money this would amount to an extra return of some 50*s*. an acre in the case of feeding corn and, in the case of the best malting barley, of no less than 150*s*. an acre. These results which concern corn infested with yellow charlock, or white charlock give, indeed, a striking picture of the damage weeds can do. They also illustrate the supreme position held by sulphuric acid as a remedy for white charlock.

I would like to suggest, however, that more information is desirable on the yield increase from more moderate infestations. To illustrate this point I will quote as a comparison the results of some weed-spraying trials carried out in Denmark by Korsmo. It was found, over a period of nine years, that the average increase by acid spraying amounted to only 4 cwt./acre. This result is of great importance to the engineer, since the average gain the farmer is likely to get from spraying determines the price he is prepared to pay for a machine. Would it be possible, by any simple field method, to gauge the intensity of weed infestation in conjunction with crop condition, so as to decide if a crop was worth spraying? I have encountered many farmers with borderline cases. They were not sure whether to spray or leave the crop alone and, almost invariably, the decision went against spraying.

To turn for a moment to an important indirect factor in the development of chemical weed destruction: as I have said, the real depreciation cost of sulphuric acid sprayers is generally excessive. By real depreciation I mean the fall in intrinsic value, as distinct from the conventional depreciation figures used in farm costing. For example, if a tractor is used but little, it will last longer. Experience with sprayers, however, indicates that their working years are numbered immediately they are used for sulphuric acid because of corrosion. The sulphates formed by the acid corrosion of brass and bronze cannot form a protective film on the parent metal as they are very soluble in the spray itself. If, then, a machine is suffering from real depreciation, whether it is used or not, we might naturally ask what other jobs can it do on an ordinary mixed farm? Normally, the answer is that it can do no other useful work. Many attempts have been made to find fresh outlets such as spraying grassland weeds, poultry land infested with coccidiosis, even the spraying of land suspected of carrying the virus of foot and mouth disease has been suggested. All these alternatives have, unfortunately, borne no practical fruit, and it has not been possible to spread the heavy cost of depreciation at all. In a meeting such as this, might I ask you to give this problem of finding alternative uses your consideration? When I said just now that no alternative work has been found for a corn sprayer I was not, strictly speaking, quite correct. As many of you know, Dr C. H. Brown has developed an entirely new use for sulphuric acid in wilting down potato haulm and chickweed to facilitate lifting of the crop. When blight is present this also prevents severe contamination of the tubers from the diseased tops. This spraying, taking place at the end of the summer, would seem an ideal opportunity for increasing the scope of a machine; but spring corn and its weeds are aggravatingly absent from the heavy rich land of the eastern counties where the great bulk of the potato crop is raised. Providence, however, has compensated for this in some small measure by providing an unorthodox use in the potato districts, where acid is also used in the spring to reduce the risk of lodging of heavy cereal crops by destroying some of the flag.

It can be said that, as a whole, the use of machines for sulphuric acid on the rather specialized potato farms to destroy haulm and chickweed is the most profitable

example of chemical spraying, on account of the heavy losses otherwise incurred in a season when blight is prevalent. This is reflected in the better and more costly equipment used there.

I want to turn now to another aspect of chemical weed destruction, namely, the reaction of the farmer. An interesting example was provided some years ago when sulphuric acid spraying was just developing in this country. On a farm in Northamptonshire a certain field, when under spring corn, was frequently almost choked by prolific growths of spurrey. The conventional remedies of liming and spring harrowing were tried without success, and when the harvest came the crop of spurrey seeds was truly amazing. The farmer began to think that perhaps he could grow spurrey more easily than corn, so he despatched a sample of the seed to a firm of chemical manufacturers to find out if it was of any use as a source of oils. A reply came back regretting that it was of no use. Nothing daunted, the farmer sent a sample to a well-known firm who manufactured bird seed, again with a plaintive enquiry. Again came a disappointing reply that the seed was of no value as a constituent even of bird seed. Then, and only then, the farmer rang us up and offered his field for experimental spraying purposes. This viewpoint suggested that spraying is regarded as the very last resource, but it is probable that with the lapse of a few more years a more favourable viewpoint will be developed.

To return for a moment to the idea of our farmer who tried to turn his spurrey infestation to financial advantage: many tons of charlock seeds are obtained in this country every harvest when spring corn is threshed, and the farmer generally burns it. To indulge in a flight of fancy, is it possible that this waste material is worthy of a biochemical investigation as a source of any useful product? I do know of one case where charlock seed is sold for 10s./cwt. and used as a constituent of food for game birds, and it is highly prized.

I want now to say a few words about the development of spraying machinery. As many of you know, within the last six years the acreage sprayed annually by sulphuric acid alone, has risen from a few hundred acres up to 30,000. In the last decade we have seen the knapsack sprayer replaced by the cart attachment, the latter in turn by the horse drawn machine with pumps driven from the axle, and to-day the latest development is the power equipped sprayer. This unit has motor driven pumps both to feed the jets and to fill the barrel and, as might be expected, is most plentiful in the potato districts where the financial return from spraying is greatest. This mechanical progress has meant a steadily increasing machine cost rising from £5 to about £100. Working rate at the same time has risen from about 1000–12000 sq. yd./hr., while manual labour has conversely decreased, falling in cost from about 4*s*. to 9*d*. an acre.

At Oxford, investigations on acid-spraying machinery have proceeded along three main lines. The first has been to improve the methods of handling acid and water in the field. One object of this was to reduce the idle time of the sprayer on the headland during filling, and has, in fact, by suitable proportioning of pump sizes, given an increase in working rate of 28% with a concurrent rise in capital outlay of 10%, which may be regarded as satisfactory.

The second benefit from improved handling methods, is to reduce risk of splashing and burning, and generally make spraying a reasonably clean job that farmers will not dislike. If, as seems possible, some producers of sulphuric acid find that contract

spraying is not remunerative, it is important that the farmer should be willing to tackle the job himself.

The second line of attack has been on the corrosion and depreciation problem. The alloy 18 : 8 nickel chrome steel has proved a resistant material under all field conditions. Unfortunately, despite its present reasonable price, no commercial machines yet embody it, as the great toughness of this alloy demands special tools and more time to machine it which, together, make it too expensive. The obvious solution to corrosion, that of cleanliness, has not proved successful in practice, partly because of the difficulty of cleaning machines properly without dismantling them.

The third line of attack has been on the water consumption. It is surprising to reflect that when a farmer sprays 50 acres of corn no less than 16–18 tons of water have to be pumped and carted. It has been observed that in the wheel tracks of a machine the weed kill is almost always 100%. This led to an experimental machine with rollers but, unfortunately, it was found that a reasonably light roller which the horses could pull did not reproduce the effect of the sprayer wheels—presumably because the ground pressure on the weeds was too low. This line is capable of development were a tractor available to pull the machine. Concurrently, the relationship between pump pressure, nozzle contour, and the size of the spray droplets, was examined. Smaller droplets may reduce the volume of spray required per acre, but will increase the draught of the sprayer by demanding an increase in the pressure drop through the jets.

To sum up, the wet sprayer still lags behind in comparison with other modern farm machinery. It is too small to take advantage of tractor power and has, consequently, a low working rate. That these technical snags can be overcome there is little doubt but, unfortunately, the bogey of capital cost, coupled with the fact that weeds are awkward irregular things, will, I think, continue to restrict further mechanical development of the sprayer that is used solely against them.

To turn now to the sphere of dry spraying or dusting; here the problem of the machine is much less acute. It may cost £35, but it has uses other than weed destruction and, consequently, its depreciation charge on any particular job is much reduced. Frequently, an existing farm distributor can be used and fresh capital outlay avoided, a factor whose value can hardly be over-estimated in farming.

We are all aware of the advantages of dry weed destruction. There are no difficulties in regard to a water supply and, concurrently, there is no serious corrosion problem. On the other hand there is not the clean and complete extinction of the weed which the wet method gives.

In part, this incomplete weed destruction is a mechanical problem, since better results are always obtained when the powder is applied evenly over the whole of the plant surfaces. This ideal is never obtained with an ordinary farm distributor, which either dresses the crop with narrow and regular bands of powder or, what is worse, with haphazard little teaspoonfuls here and there, giving one weed too much and another no powder at all. Distribution which is perfectly uniform when expressed as weight of powder/sq. yd. can be hopelessly irregular when expressed as weight/sq. in.

Frequently, however, by fitting to a machine little paddles that break up the falling streams of powder, much more uniform results can be obtained, at an additional expenditure of about £5.

The working rate of a typical distributor is about the same as an axle driven sprayer, but the distributor can only work effectively for 3–5 hr. a day while the dew is on the weeds.

The cost of applying dry powders such as cyanamide in this manner is about 1*s*. 6*d*. per acre, while to apply acid costs about 4*s*. 6*d*. and possibly more. The overall cost of the wet and dry methods are roughly the same, however, as the acid costs less than the cyanamide. The cyanamide, of course, gives the crop about 100 lb. of lime and 30 lb. of nitrogen to the acre, but the latter constituent is, unfortunately, not often wanted by the grower of malting barley.

Within the last five years equipment for dry spraying has advanced considerably and, by dispersing powders in air before applying them to the crop, much better weed destruction is attained. In addition, machines are available that can work at the rate of about 50,000 sq. yd./hr., far surpassing any wet sprayer in output.

III. THE RELATIVE TOXICITY OF CHEMICAL WEEDKILLERS

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During the last 30 years a large number of substances have been tested for their effectiveness in destroying unwanted plants. To-day the compounds which are most widely employed in agriculture and horticulture can, in regard to the nature of their action, be divided into three categories. There are, firstly, compounds such as sulphuric acid or sodium hydroxide which are only successful when applied in comparatively strong concentrations, since their destructive power is dependent on the solution being either strongly acid or alkaline. The second group, which is by far the largest, contains compounds which are effective in dilute concentrations, since they or their ions accumulate in toxic amounts within the plant. In the third group, the compounds are neither directly toxic in dilute concentration nor are the solutions acid or alkaline. Their action is dependent on a high solubility in water and, in consequence, plasmolysis of the plant tissues. I do not propose to discuss in any detail the comparative effectiveness of substances either in the first or last group. Recent research has indicated, at least where the acid substances are concerned, that they are equally efficient as long as the hydrogen ion concentration is of the same order. But, in the second group, our knowledge of relative toxicity is far less certain for, in the past, few experiments have been carried out in which several compounds have been tested simultaneously under a wide range of conditions.

In attempting to assess the merits of various compounds several difficulties confront the experimenter. It has been shown repeatedly that species vary enormously in their susceptibility to any one compound. For example, we have found at Jealott's Hill that, even within a single genus, there are wide divergencies. A dressing of sodium chlorate at the rate of 60 lb./acre will almost completely eradicate *Plantago coronopus* and *P. lanceolata* from pastureland. Yet the same dressing will only partially control *P. major*, while *P. media* is completely resistant. Again, in some species the season of the year must be taken into account. For the control of *Cnicus*

arvensis an autumn application of sodium chlorate is more effective than a spring one, but in the case of *Hypochaeris radicata* the time of application is immaterial. Moreover, weather conditions must also be borne in mind. Copper sulphate is ineffective for the eradication of *Brassica arvensis* in showery weather, while sodium arsenite sprays give the best results during times of drought.

In order, therefore, to obtain a true comparison between several substances experiments were carried out over a range of both season and weather conditions. The chief plant investigated was *Senecio Jacobaea*, since there was evidence that it was susceptible to a member of the more well-known weedkillers. Among the substances tested were aluminium sulphate, aluminium nitrate, ammonium thiocyanate, copper nitrate, copper sulphate, sodium arsenite, sodium bichromate, sodium bisulphite and sodium chlorate. The compounds were either applied in the autumn in powder form (sodium arsenite in solution), or as sprays (100 gal./acre) at different times during the growing season. The first experiments demonstrated that the aluminium salts, the copper salts, sodium bisulphite and ammonium thiocyanate were all entirely ineffective. In the later experiments these, with the exception of ammonium thiocyanate, were omitted. These later experiments, however, confirmed the earlier results for ammonium thiocyanate since even when the rate of application was as high as 300 lb./acre only 20% of the plants were killed. Of the remaining three compounds, sodium chlorate, sodium bichromate and sodium arsenite, the first gave by far the best results. Irrespective of either the season or the weather conditions, sodium chlorate at a rate of 50–60 lb./acre gave a control of 88–100%. Sodium bichromate was only effective at much higher concentrations; complete suppression was not obtained until the rate was raised to 300 lb./acre. The results for sodium arsenite were very variable; applied in amounts equivalent to 13–15 lb. of arsenic trioxide per acre the control ranged from 0 to 72%. The efficiency was greatest during dry weather when the plants showed signs of wilting. The effectiveness of sodium arsenite and other compounds was not increased by the addition of wetters to the spray.

While these experiments have established for *S. Jacobaea* that sodium chlorate is the most toxic of the weedkillers tested, it does not necessarily follow that this result holds for other species. Only further experiments along the lines of this investigation can settle whether the chlorates are the most effective weedkillers for other plants.

IV. THE CONTROL OF WEEDS IN LAWNS AND FINE TURF

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In the space allotted in this symposium it is only possible to cover the field in general terms but it cannot be covered adequately by describing chemical methods of control only, because good management demands various supplementary operations some of which are mechanical. It should be realized also that turf weeds are not solely non-gramineous but that a number of pernicious weed pests are grasses. Furthermore the establishment of new turf in a weed free condition, the prevention of weed invasion, the use of grass species or strains which are aggressive enough to resist weed invasion,

are all important aspects of this problem. Weed invasion and weed eradication are closely related but there is no single all embracing method which can be advised to suit all conditions. Perhaps the problem may best be understood by first of all giving a brief summary of the requirements of a good turf.

Requirements

A good turf must be dense, uniform in texture and true because trueness means even mowing and thus greater uniformity. If the turf consists of more than one species then even blending is important. The surface should be carpet-like and there should be some resiliency to the foot. To be successful the sward should consist of dwarf, fine-bladed grasses, capable of making bottom growth and it should consist predominantly of species of bent (*Agrostis*) and fescue (*Festuca*). To produce this state of perfection demands intensive management. The commonness of bad turf areas is due to neglect and a failure to appreciate the amount of work necessary to produce the high degree of excellence desired by many but attained by few.

Of all the operations of maintenance, mowing is perhaps the most abused. Mowing determines very largely the species of grass in a turf and it is because of the mowing that the weed problem often becomes so acute. In the finest lawns mowing three times per week is not excessive and many modern golf courses arrange for mowing to be carried out seven days a week in the height of the season. The constant defoliation removes competition and enables weeds to become established, many of which are able not only to persist but rapidly increase under conditions of keen mowing.

Four classes of weeds may be considered:

(1) The mat weeds consisting of such species as pearlwort, mouse-ear chickweed, yarrow, selfheal, creeping buttercup and bedstraw, all of which spread by runners and form a dense mat. The mosses are perhaps best included in this group.

(2) The flat or rosette weeds containing for example four species of plaintain, daisy, cat's-ear, and the erodiums, some of which spread by budding out daughter plants.

(3) The clovers, principally creeping clover and yellow suckling clover (the latter spreading by seeding).

(4) The annuals, parsley piert, shepherd's purse, chickweed, groundsel, peculiar to new lawns, certain grasses such as Yorkshire fog, annual meadow-grass, perennial rye-grass, and other coarse species.

Few annuals persist in regularly mown turf, the exceptions being such plants as annual meadow grass and parsley piert which flower and set seed below the level of the mower blade.

Although mowing may be a primary factor in determining the species of plants able to persist in fine turf, the species of grass present and even the strain have an influence in determining the incidence. A dense matted species inhibits invasion to a large extent; furthermore soil factors are partly responsible. The effect of species is shown by the following figures for plots of the same age and treated alike:

Chewing's fescue (*F. fallax*) plot: 63% grass, 19% weeds.
Browntop (*A. tenuis*) plot: 59% grass, 9½% weeds.

New lawns

Faulty establishment of new lawns is often the cause of future trouble and perhaps the commonest error is insufficient fallowing to secure a clean seed bed. Impure seed is also another source of trouble, and failure to sow uniformly may assist weed entry. The seed rate when sowing a new lawn may be from 3 to 6 cwt./acre, and all efforts must be directed to secure a rapid and uniform establishment of the grass by thorough cultivation and pre-treatment of the soil so as to ensure a density sufficient to keep down any surviving annual weeds.

In view of the high seed rate the use of pure seed only is exceedingly important; for instance a sample of Chewing's fescue seed containing 2·5% of grass and non-grass impurities when sown resulted in the sward being only 75% pure with 22% of grass weeds and 3% non-grass weeds. A similar sowing using browntop seed containing 1·6% of grass impurities resulted in a turf only 63% pure with 27·5% of Yorkshire fog and other grass weeds, and 9·5% bare ground.

The importance of clean seed may be realized by expressing the matter in another way. Thus, if browntop containing 0·1% of mouse-ear chickweed is being sown at 2 oz./sq. yd. (about 6 cwt./acre), there will be approximately 550 seeds sown on each square yard. Again if 2 oz./sq. yd. of Chewing's fescue containing 0·2% perennial rye-grass is sown to the square yard, then 57 seeds would also be sown. The presence of even one or two plants of perennial rye-grass per square yard in a fine turf is sufficient to cause considerable expense in eradication on anything but very small areas. It is not entirely the weight of weed seed present but their number and nature as well as their potential danger which decides whether or not the grass seed is suitable for use.

Many new lawns are established with rye-grass mixtures and weed invasion is usually rapid since rye-grass rarely forms a dense turf. Where a percentage of browntop is included bottom growth is formed which then appears to accelerate the gradual decline of the rye-grass under conditions of keen mowing. Thus a plot sown with indigenous perennial rye-grass, smooth stalked meadow-grass, and crested dogstail, consisted after 4 years of 45% rye-grass, 21% other grasses, 26% weeds, whilst the adjacent plot sown with the same seeds and 50% of browntop, contained at the end of the same period only 5% rye-grass, 5% other grasses, 71% bent, and 15% weeds. In new sown turf where the soil has been poorly cleaned the occurrence of annual weeds like chickweed, shepherd's purse and groundsel is not uncommon. Provided these weeds are not too extensive, they gradually die out as a result of regular mowing though in some cases with autumn sowings chickweed may seriously assert itself in the interval before mowing is possible the following spring.

Established lawns

Having established a turf of pure seed it is necessary that treatment should be directed to maintaining it in weed free condition. Much attention has been devoted to this problem in the United States and the work has very largely been repeated at St Ives with substantially the same results. For example, by regularly fertilizing pure sowings of Chewing's fescue and browntop, with ammonium sulphate, chloride, or phosphate, or with sulphate of ammonia plus superphosphate with or without potash, it has been possible to maintain the turf in weed free condition over a period of

8 years. The addition of sulphate of iron assists the process. On the other hand the effects of the continuous use of nitrate of soda, nitro-chalk and nitrate of lime, and the above ammonium fertilizers with lime has led to the invasion of other grass species and weeds. Where Yorkshire fog is present however it is able to persist under mown conditions even when ammonium compounds are used.

Examples (Table I) from the botanical analyses taken in 1937, on plots started in 1929 may be of interest.

Table I

Plot no.	Species sown	Treatment	Year started	% fescue	% other grass	% weed
1	Fine-leaved fescue	S/A S/I	1929	99·5	0·0	0·5
2	,,	S/A S/I lime	1929	3·0	60·5	36·5
7	,,	P.K. S/A	1929	91·0	9·0	0·0
8	,,	P.K. S/A + lime	1929	0·0	18·5	81·5
1	Chewing's fescue	No fertilizer	1929	52·5	6·0	41·5*
2	,,	S/A	1929	89·5	10·5†	0·0

S/A = sulphate of ammonia.
S/I = sulphate of iron (calcined).
* = containing 37% moss.
† = *Holcus* sp. and *Agrostis* sp.

Effects of mowing

As already mentioned, the general effect of mowing is to remove the competition of the grass and to permit weed invasion. The return or removal of the cuttings on each occasion also has an effect on the nature of the sward. Thus, on an area to which the cuttings are always returned the amount of annual meadow-grass tends to increase and stands at 22%, as against the adjacent plot from which the cuttings are always removed where the percentage of this grass is only 2%. No doubt this difference is largely due to the return of seeds from the panicles of the annual meadow-grass but the softer and moister soil where the cuttings are returned may also favour this volunteer species.

Experiments are in progress at St Ives to determine the effect of height of cut (three levels) on weed invasion and upon the general condition of the turf. This work still continues, but it has been found that closer cuts give a denser turf and, indeed, with the longer cuts, the amount of weed tends to increase within the limits of the experiment.

Table II shows the comparisons of weed percentages (1937 figures) on certain of the plots started in 1931.

Table II

Treatment	Height of cut inches	% weeds	% moss	% grass	% bare ground
N/C + compost	$\frac{1}{4}$	2·5	19·0	78·0	0·5
N/C + compost	$\frac{3}{4}$	23·0	0·0	70·5	6·5
S/A + compost	$\frac{1}{4}$	0·0	1·5	98·0	0·5
S/A + compost	$\frac{3}{4}$	0·0	0·0	98·0	2·0
Compost only	$\frac{1}{4}$	1·5	34·0	64·5	0·0
Compost only	$\frac{3}{4}$	8·5	11·0	80·0	0·5

It will be noted that on the shorter mown plots there is a higher percentage of moss even though the number of shoots per unit area has been found to be greater and, possibly, this may be correlated with the degree of shading.

Experiments on weed eradication

A brief consideration may now be given to weed eradication as distinct from weed control or inhibition. In 1931 an area of weedy turf was subdivided for various manurial treatments and a series of nitrogenous dressings applied, both with and without sulphate of iron since previous work had shown that the effect of adding sulphate of iron to nitrogenous manures was to accelerate the reduction of weeds. It was found that all forms of nitrogen lead to a reduction in the weed population but the largest reduction, which in most cases was complete, took place with sulphate of ammonia and ammonium phosphate and least with dried blood, urea, and cyanamide, whilst nitro-chalk and nitrate of soda came in between. The addition of sulphate of iron even to materials like nitro-chalk and nitrate of soda leads to increased weed reduction, and the use of sulphate of iron alone in amounts considerably greater than those used with fertilizers results in marked reduction of such species as daisy, ribwort plantain and selfheal. A full account of this work is in preparation.

Other experiments have been specifically concerned with the control of clover in fine turf, and Table III shows the effects of certain regular treatments started in Autumn 1931.

Table III

Treatment	% weeds	% clover	% moss	% grass
Control	2	22·0	32·0	44
S/A compost	0	T.	3·0	97
N/S compost	6	0·5	32·5	61
S/I only	0	34·0	8·0	58

N/S = nitrate of soda.
T. = trace.

The figures shown in Table III were obtained in 1937 but are substantially the same as those secured in 1933. It will be noted again that the sulphate of ammonia has a more striking effect than nitrate of soda, whilst on the sulphate of iron plot there is more clover than on the control. This may be due to the fact that regular use of sulphate of iron leads to a thin open turf and conditions more suitable for the spread of this weed.

Practical methods of weed eradication

Practical methods of weed eradication nearly always entail the use of sulphate of ammonia or ammonium phosphate with or without sulphate of iron, and if there is a foundation of fine grass, then the use of these materials will, after a number of dressings, result in rapid elimination.

The common formula used on lawns consists of:

3 parts by weight sulphate of ammonia.
1 part by weight sulphate of iron (calcined or fine crystals).
20 parts by weight carrier (such as potting soil or compost).

This mixture is applied six times per annum at 4 oz./sq. yd. This mixture is particularly effective against daisy, selfheal, pearlwort, and chickweed but, for dealing with cat's-ear, dandelion, and plaintain, a more concentrated mixture is applied. This consists of:

3 parts by weight sulphate of ammonia.
2 parts by weight sulphate of iron.
5 parts by weight carrier such as dry sand.

This mixture is used broadcast at 3–4 oz./sq. yd. or as a spot treatment. Ammonium compounds give a fair degree of selectivity, and if careful application is made damage to the grass is, usually, only temporary. Weed control by this process may be attributed to a combination of factors, for example nitrogen effect on the grass, increased soil acidity, direct plasmolysis and ammonium toxicity.

For weeds like moss and pearlwort, larger quantities of sulphate of iron are usually employed and a mixture of equal parts of dried blood and sulphate of iron is commonly used for controlling pearlwort. For moss control careful raking, followed by improvement of the fertility of the soil is adopted since moss is usually a sign of neglect and poverty rather than of dampness. Forking plus light liming where tests indicate, are often supplementary but at times attention to drainage may be necessary. Extensive experiments have been carried out with permanganate of potash at St Ives for moss control. Reduction is mainly temporary unless very heavy rates are used, the cost then being 5 or 6 times more than for sulphate of iron.

For large scale work on playing fields, large lawns, and gold fairways, sulphate of ammonia and sulphate of iron are commonly used without carrier; a mixture of 3 or 4 to 1 at 1–2 oz./sq. yd. applied with special types of distributors is very efficient. Other lines of work which are being investigated, following work in New Zealand, are spraying with arsenic acid at strengths from 1 in 60 to 1 in 120, according to the species of weed concerned.

For controlling annual meadow grass, dilute arsenic acid spraying appears to have possibilities though it has the disadvantage of causing a complete browning of the turf. Where weeds are extensive, renovation after this method is of course necessary and, indeed, on any area where the weeds have been excessive.

Experiments with chlorates and thiocyanates have been carried out but have not been sufficiently striking to warrant extensive work. More recently solid cones containing sodium chlorate have been put on the market and these are inserted into the crowns of the weeds. For rosette weeds they are very efficient and damage to the surrounding turf is kept to a minimum. Other large scale methods of killing rosette weeds on golf courses entail the injection of dilute arsenic acid into the crowns of the weeds and, by applying a few drops of the acid, through an oil-can, to the centre of the rosette.

As already mentioned, the control of weeds in fine turf does not involve solely the use of chemicals, since mechanical operations are frequently necessary either alone or supplementary to chemical treatment. Thus, for moss, occasional raking is desirable and, for the control of Yorkshire fog since no selective chemical method of eradication is yet known, the present method is to carry out systematic slashing followed by renovation with fine seeds and an improvement of the fertility. The raking of all mat weeds is desirable and particularly is this so in the case of creeping clover, the runners of which must always be raken up and removed with the mower. For weeds like chick-

weed constant use of a drag brush on the turf prior to mowing is an advantage, and to control annual meadow-grass drag brushing prior to mowing is beneficial. The same treatment has been adopted successfully for the control of rough-stalked meadow-grass. Mechanical operations are also useful in controlling yarrow which may be closely shaved by a scythe or special hoe, made for the purpose, and also by slashing the stems and underground parts with special slitting tools. Close "shaving" of clover in hot weather followed by light applications of sulphate of ammonia is effective in reducing this weed on most soils.

V. CHLORATE WEEDKILLERS

By O. Owen, M.Sc., Ph.D., A.I.C.

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Whilst previous contributors have dealt with the efficacy of various chemicals as weedkillers this contribution deals briefly with injury which has resulted from the careless use of a very popular weedkiller, namely sodium chlorate. Except for the fire risk involved in its use sodium chlorate appears to have all the properties of the ideal weedkiller, and this has led to its use on an increasing scale in nursery and market garden work. Its high toxicity and the fact that it is not specific in its action have, on many occasions, caused nurserymen serious losses.

The first case which was brought to our notice concerned a tomato nursery in which a large number of plants were affected. The foliage showed a definite mottle, older leaves were desiccated and brown lesions were apparent on the stems: the symptoms generally were those associated with what was then known as "stripe" disease. The effects were confined to two well-defined areas in two houses. The application of fertilizers and repeated planting had been of no avail. Eventually the injury was found to be due to chlorate in the soil, the concentrations varying from 0·02 to 0·03% calculated as sodium chlorate. Steam sterilization caused a definite improvement in the condition but the beneficial effect was not permanent, and it was not until two years later that the injurious effects had completely disappeared: this despite very heavy floodings.

Since then cases dealt with have been many and varied; at some time or another practically all the plants raised in nursery work have suffered.

A common instance is that in which paths outside tomato houses have been treated with chlorate for the eradication of weeds. Rows of plants adjacent to the boundary walls often show effects of chlorate poisoning. An interesting case in this connexion is one where a group of plants 12 ft. away from a wall were showing mild but definite symptoms of injury. The paths outside the house had been treated six weeks previously. The plants showed ill-effects within a few days of the first heavy rainfall since the chlorate had been applied.

Another source of danger is that which attends the growing practice of treating "standing-out" ground with chlorate. This may cause injury in two ways. In the first the pots may become contaminated without actually affecting the plants in them, e.g. heaths which are comparatively resistant to chlorate. When, however, the pots are brought into houses enough chlorate may be washed off the pots into the soil to injure a succeeding crop, such as tomatoes, planted in the soil. Then, of course the pots may themselves be used for other plants of a higher susceptibility with con-

sequent damage. There is, also, the great risk that plants such as chrysanthemums may be affected. We had one case of extensive injury on a nursery where chlorate had been used for some years with satisfactory results. The damage reported was found to be due to the fact that a workman had omitted to wash in the weedkiller which had been applied in the dry state. Another case of chrysanthemum injury concerned a grower who had treated the "standing-out" ground in the spring of 1936. Later in the year 4000 chrysanthemums were stood out and proved a complete failure. In the late autumn the grass on the ground was quite green again and in 1937 it was cut two or three times but again the crop was a total loss. The pots had taken up and retained sufficient chlorate to injure the plants.

It is interesting to note that injury to chrysanthemums is often confined to the tops where the leaves become a peculiar grey-green colour, with considerable distortion and some shrinking, while the lower leaves exhibit little or no abnormality.

One other case may be of interest. Many crops in a kitchen garden were thought to be suffering from a virus disease, but examination of the soil showed the presence of a chlorate despite repeated assurances that no chlorates had been used near the garden in question, nor had any watering cans been used for applying chlorate. Eventually it was found that water was drawn from a pond in another part of the garden and this water had become contaminated by drainage from paths which had been treated with chlorate.

There is a definite variation in the susceptibility of different cultivated plants to chlorates. Of those met with here, heaths definitely show the least susceptibility. At the other end of the scale tomato plants, dahlias and winter cherries are among the most susceptible. During sunny weather under glass a young soft, tomato plant will show signs of injury well within 24 hr., with concentrations of the order of one part of sodium chlorate per ten thousand of soil.

In addition to the plants already mentioned the following plants have, among others, been found to be suffering from chlorate injury: garden nasturtiums, wall flowers, cinerareas, asters, cucumbers, lettuce, antirrhinums, primulas, geraniums, sweet peas.

Generally, where the poisoning is mild the plants show a distinct mottle which may be (and actually has been) mistaken for a virus effect. Where injury is more severe, distortion of the foliage and desiccation occur. More detailed information concerning effects and concentrations producing them will be found in the *J. Minist. Agric.*, 1937, **44**, No. 6, p. 866.

REVIEWS

Introduction to Plant Pathology. By F. D. HEALD. Pp. xi + 579. London: McGraw-Hill Publishing Co., Ltd. 1937. 24*s*. 0*d*.

In 1933 Prof. Heald published a second edition of his *Manual of Plant Diseases*, a standard American treatise running to 953 pages, costing 45*s*., and intended for serious students. A shorter, cheaper, and more elementary introduction to the subject has been needed and Prof. Heald has filled the gap. The new volume resembles the *Manual* in many ways, but it is not a mere abridgement; the material has been rewritten, new matter is included, and there is a different order of presentation.

The book is divided into six sections, the first being introductory. Ch. I deals briefly with the nature of plant disease and the development of the subject, mostly in America. Ch. II is an excellent summary of the symptoms of disease in plants, although one would have liked to see this subject treated more on a developmental basis. Ch. III discusses the relation of fungi and bacteria generally to human affairs. This is an interesting innovation and contains much useful material, but some of it seems a little out of place in a text-book specifically of plant pathology. Ch. IV deals with the nature and extent of plant disease losses. Ch. V concerns the dissemination of plant-diseases.

Section II, devoted to the parasitic diseases, comprises ten chapters. Ch. VI introduces the student to the vegetative and reproductive structures of fungi, and the following nine chapters deal respectively with diseases due to Phycomycetes, Ascomycetes, Ustilaginales, Uredinales, Hymenomycetes, Fungi imperfecti, bacteria, parasitic seed plants, and nematodes. The general method of treatment in each chapter is to outline the structure and classification of the particular group, describe selected diseases in some detail, and then tabulate the diseases and parasites. Thus, Ch. VIII contains two introductory pages on the Ascomycetes; detailed considerations of peach leaf curl, brown rot, anthracnose of currant, powdery mildew of apple, ergot, black knot, and apple scab; and a table of 121 important diseases due to Ascomycetes. A more or less standard mode of presentation is adopted for each disease: history and distribution, symptoms and effects, crop losses, etiology, pathological anatomy, climatic relations and predisposing factors, host relations and varietal resistance, prevention and control, references. The selection and ordering of the material in these chapters is excellent.

Section III comprises two chapters on viruses and virus diseases. The first deals generally with the types of viruses, methods of transmission, and the nature of viruses; and the second contains descriptions of specific virus diseases, and a list of 241 diseases of 141 host plants. Section IV is devoted to the non-parasitic diseases, which are discussed in four excellent chapters as follows: diseases due to unfavourable soil conditions, and deficiencies or excesses of food materials; diseases due to improper air, temperature, and light relations; diseases caused by manufacturing or industrial processes; and diseases due to control practices. Section V concerns the prevention and control of plant disease and, following an introductory chapter, three chapters are devoted respectively to disinfesting practices, sanitary and cultural practices, and seed selection and the selection and breeding of disease-resistant varieties. Section VI deals briefly with phytopathological technique and contains three chapters devoted, respectively, to methods of studying parasitic, virus, and non-parasitic diseases. The book opens with a synoptic contents, and closes with an index which omits reference to diseases and parasites merely listed at the ends of chapters.

The selection and arrangement of the material and the balance of the sections seem to me admirable, and although the volume is arranged on a mycological and not a host basis it is a real introduction to plant pathology and not merely a text-book of semi-applied mycology. The author's style is clear and simple, but he is not entirely

consistent in his terminology and, occasionally, e.g. at the top of p. 351, his pen runs away with him. The book contains many Americanized spellings, and misprints have been noted on pp. 19, 39, 56, 114, 160, 163, 217, 249, 270, 301, 497, and 566. Students may find the bibliographies annoying, since, for investigations published before 1932, reference is merely to the larger *Manual*. The 200 illustrations are of good quality and well chosen, but there seems no reason for the waste of space on pp. 235–7. As the book is written for American students, American diseases, naturally, are emphasized, and certain diseases which would find an important place in an equivalent English course are either omitted or merely noted; e.g. silver leaf of plum, wart disease and powdery scab of potato, yellow rust and whiteheads of wheat, leaf stripe of barley, clover rot, chocolate spot of bean, American gooseberry mildew, spotted wilt of tomato, reversion of black currant, etc. Other matters of interest are the use of small letters for all specific names, the adoption of the genus *Monilinia* for the monilioid pseudosclerotial *Sclerotinias*, and the inclusion of *Plasmodiophora* and *Spongospora* in the Chytridiales, and *Actinomyces* in the Hyphomycetales.

Prof. Heald is to be congratulated on having written perhaps the best *Introduction to Plant Pathology* yet published.

WILLIAM B. BRIERLEY.

Der Schwarzrost: seine Geschichte, seine Biologie, und seine Bekämpfung in Verbindung mit der Berberitzenfrage. By E. LEHMANN, H. KUMMER and H. DANNENMANN. Pp. xxiv + 584, 1 colour plate and 87 text-figures. Munchen/Berlin: J. F. Lehmanns Verlag, 1937. Rmk. 21 (cloth-bound); Rmk. 19.50 (paper covers).

There are numerous books on general plant pathology, on the diseases caused by one or another group of pathogenic agencies, on the diseases of particular crop plants, or on particular groups or genera of parasites, but the present book is something new in plant pathological literature in that it is a treatise of very respectable size devoted entirely to one disease. The only other publications of somewhat similar nature I am acquainted with are McAlpine's work on bitter pit of apple, and Falck's work on dry rot of timber, but these are more progress reports than complete and independent treatises. The publication of *Der Schwarzrost* sets a new standard in plant pathology and, in a way, marks the commencement of a new period; a period of specialization on individual diseases rather than on individual crop plants, or individual genera or groups of parasites; one hopes that the present work may be but the first of a series of such monographs. So commanding a volume could hardly be written on any other single disease, since black rust is probably the most important plant disease in the world, but potato blight might run it very closely and shorter monographs of similar type on, e.g. club root of crucifers, wart disease of potatoes, downy mildew of the vine, apple scab, ergot, bunt of wheat, silver leaf, crown gall, fire blight, low or high temperature injury, etc., are immediately feasible and would be of great value.

Black rust of wheat has received an enormous amount of attention, as may be gauged from the fact that the Bibliography in the present volume contains some 2000 references in all sorts of languages and, even so, it is not complete. The reviewing of this mass of work, which is very heterogeneous and of uneven quality, and the ordering and considering of the data must have been a Herculean task. The authors have done their work admirably but their very conscientiousness has led them into perhaps unnecessary length and, here and there, a little more severe and critical handling of some of the work might have been desirable. Every facet of the subject is considered in detail but, in reading the book, one never gets a sense of undue padding or of false values.

Ch. I is a mere introductory note. Ch. II (37 pages), is an interesting historical survey of the subject, commencing with ancient biblical times and ending with biotypes: it is also, to a certain extent, a very brief summary of the book itself.

Ch. III (27 pages), concerns the world distribution of the barberry, and Ch. IV (47 pages), its indictment in various countries. Some of the matter in these two chapters seems a little unnecessary, but the detailed consideration of the barberry may be justified by the fact that, in central and western European countries far more than in many other wheat regions of the world, the barberry retains its primary importance as an obligatory alternate host of the parasite in relation to outbreaks of black rust. Ch. V (228 pages), is a very detailed consideration of the biology and physiology of parasitism of *Puccinia graminis* in both graminaceous hosts and the barberry. Every phase of the problem is dealt with in a remarkably efficient way, and these pages contain the most thorough discussion by far of any particular aspect of disease I am acquainted with in plant pathological literature. They are, as it were, a complete "Fischer & Gäumann" of black rust. In Ch. VI (27 pages), the epidemiology of black rust is considered and, although some first class work has been done on this problem, especially in America, the inadequacy of our knowledge is very evident. It is a curious fact that, although plant diseases are especially suitable material for epidemiological study, yet, epidemiology remains one of the most neglected aspects of plant pathology. Ch. VII (40 pages), gives an account of the world distribution of black rust, and of the available but inadequate data on the damage it causes. Ch. VIII (106 pages), is an excellent account, with especial reference to barberry eradication, of the practical measures which have been adopted in various countries to control black rust. The book closes with a useful Bibliography (57 pages) and a somewhat slight Index.

So densely packed and encyclopaedic a treatise might easily have lost much of its value by inefficient arrangement of material but, fortunately, the work is well organized and a detailed Contents, running to 14 pages, makes it easy to find any particular information one needs. This book is a work of quite outstanding merit, conceived and written in the older and greater German scientific tradition. It sets a standard for all monographs of similar nature on plant diseases, and it will long remain the primary source of knowledge and inspiration for workers on black rust.

WILLIAM B. BRIERLEY.

The Structure and Development of the Fungi. By H. C. I. GWYNNE-VAUGHAN and B. BARNES. 2nd edition. Pp. xvi+449, with a Frontispiece and 309 text-figures. Cambridge: University Press. 1937. 18*s*.

The first edition of this well-known book, published in 1927, received notice in the *Annals* 1928, **15**, 150. The new book shows slight rearrangements but follows closely the lines laid down in the first edition: two short introductory chapters on general fungal morphology and classification, and on fungal physiology and forms resembling fungi; three long chapters containing a detailed consideration of the Phycomycetes, Ascomycetes and Basidiomycetes; two pages on the Fungi imperfecti; a short chapter on mycological technique; a bibliography and an index.

During the period between the editions the whole front of mycology has advanced: the details of the life histories of numerous fungi have been elucidated, heterothallism has been discovered in the rusts and found to be of widespread occurrence in the fungi generally, and there has been greater realization of the importance of flagellation as a guide to interrelationships in the Phycomycetes. This progress is reflected in almost every page of the book, the new knowledge having been digested and assimilated skilfully in the present text. As in the first edition, the authors are severely logical in their treatment and conservative in their outlook; the book is a model of condensation. The number of illustrations has increased from 285 to 309, the bibliography from 29 to 42 pages, and the index from 18 to 25 pages.

The new edition will enhance the high reputation of its authors and receive a warm welcome from all academic students of the fungi.

WILLIAM B. BRIERLEY.

A First List of Cyprus Fungi. By R. M. NATTRASS. Pp. xvi+87. Nicosia: The Government of Cyprus. 1937. 2*s*.

The introduction contains a brief account of the topography, meteorology and vegetation of Cyprus, with general notes on the relative incidence and distribution of the fungi. The list comprises 351 named organisms classified as follows: Bacteria, 5; Archimycetes, 1; Phycomycetes, 20; Ascomycetes, 37; Ustilaginales, 19; Uredinales, 91; Hymenomycetes, 35; Hyphomycetes, 87; Melanconiales, 15; Sphaeropsidales, 41. There are six new species with Latin diagnoses, two new combinations, and one new variety. The fungi are mostly pathogenic, and a portion of each collection, on which identification is based, has been deposited in the Imperial Mycological Institute, Kew. The fungi are listed alphabetically within their respective groups, with notes on spore measurements, locality, pathogenicity and host range. There are a host index of 236 plants with their fungal parasites; a short bibliography; 15 plates with drawings of the fructifications and spores of 49 fungi; and a topographical map of Cyprus showing crop regions. The genus *Acrostalagmus* is retained; *Hormodendrum cladosporioides* is listed as a fungus distinct from *Cladosporium herbarum*; small letters are used for all specific names; and there are occasional wrong spellings, e.g. "*Beauvaria*" for *Beauveria*.

WILLIAM. B. BRIERLEY.

(1) *An Introduction to Economic Botany.* By J. GILLESPIE. Pp. 96. 1937. 1*s*. 6*d*.

(2) *An Interpretation of Biology.* By H. COLLIER. Pp. 96. 1938. 1*s*. 6*d*. London: Victor Gollancz, Ltd.

These are volumes III and VII, of "The New People's Library". The publisher states: "The aim has been that each book (*a*) should be authoritative, (*b*) should be simply written, (*c*) should assume no previous knowledge on the part of the reader."

Mr Gillespie's book is not concerned with the subjects usually implied by the term "economic botany": it is an introduction to general botany and to some of its applications in agricultural and horticultural practice. The subject matter is not too well arranged and contains occasional inaccuracies and ambiguities.

Mr Collier's book is a rather polemic introduction to human biology.

Both books are written in a colloquial style and illustrated by unnecessarily crude diagrams.

WILLIAM B. BRIERLEY.

Farm and Garden Seeds. By S. P. MERCER. Pp. 205. 1938. 10*s*. 6*d*.

In view of the importance to the farmer and grower of seed testing and weed seed impurities, and of the fact that seeds legislation has been in existence in this country since 1869, it is surprising that so little has been written specifically upon this subject: "Parkinson & Smith", "Remington", and odd chapters and pages in Percival's *Agricultural Botany*, Fream's *Elements*, Hunter's *Encyclopaedia*, and in other general works. On the continent of Europe the subject has received much more the attention it deserves.

The author of the present book, the Head of the Seed Testing Division in Northern Ireland, describes his aim as follows: "This book essays the rather delicate task of giving the non-technical an outline idea of the beautiful mechanism of reproduction in plants, setting before the agricultural student a syllabus of essentials to his seed studies, offering a skeleton guide to the professional business of seed testing, and indicating to the farmer or gardener the means available to him for cheap insurance of braird." The book opens with a simple account of the nature and development of

seeds (24 pages), and describes the various aspects of commercial seed production (33 pages), and the aims, technique and interpretation of the results of seed testing (40 pages). Crop and weed seeds are then described (47 pages) and, in a final chapter, (23 pages) Mr A. W. Munro of the Ministry of Agriculture and Fisheries gives a brief but useful account of The Seeds Act, 1920; its provisions and administration. There are two appendices containing, respectively, physical data of crop seeds, and notes and data for practical seed testing. The book closes with an index. There are four pages of text-figures and 15 plates, ten of the latter containing accurate and rather beautiful pencil drawings by the author of numerous crop and weed seeds as seen under a ×12 pocket lens. The plates are not numbered but their contents are numbered in a tiresome manner. The author writes in an attractive style, his description of seed testing apparatus and procedure are brief but adequate, and his verbal portraits of seeds cogent and apt. He is a little irregular in his use of small and capital letters for specific names.

WILLIAM B. BRIERLEY.

General Plant Physiology. By E. C. BARTON WRIGHT. Pp. 539, 44 figures. London: Williams and Norgate, Ltd. 15*s.*

It is indicative of the new outlook on plant physiology as the dynamic branch of botany that after many years in which the only standard text-book on the subject was Palladin, revised and re-revised by Livingston, three text-books of major importance should have appeared in as many years. The tremendous strides which physiology has made in the last ten years, largely as a result of the new viewpoint which makes the study of the living plant as a co-ordinated entity the principal object, have emphasized the need for books presenting the subject from this modern angle.

Unfortunately, it is in just this respect that the work under review "misses the bus." The lay-out, the order of presentation and the whole conception of the book are admirable, but it fails in balance, critical exposition and modernity. It bears the marks of a work, the greater part of which was written some ten years earlier but left unpublished, and then issued with addenda. An analysis of the citations shows that out of nearly 700 references less than 18% are to papers dated 1930 or later, and many of these are referred to in the text only by brief paragraphs at the end of a section or chapter. As a result, recent work and views receive much less than their full share of attention and much is wholly omitted. For example, the work of Bolas and Goodall on the relation of assimilation to environmental factors, and that of Gregory and of Purvis on the physiological aspects of vernalization are not even mentioned, and these are only two of many cases of omission of really fundamental research of recent years.

As to the charge of lack of balance a single example will suffice. Nearly seven pages are devoted to an account of the controversy on mycorrhiza, the arguments and counter-arguments being set out in considerable detail, while the subject of dormancy in seeds, which is far more important physiologically, is dealt with inadequately in four pages, and dormancy of buds receives no mention at all despite the mass of literature which has been published on the subject.

As a text-book for students, however, perhaps the most serious criticism to be levelled at the book is that it is not a critical digest. Facts and theories are presented with no indications in many instances of the author's personal views. To be of use to first or second year students a work of the kind must be more than a text-book, it must be a guide-book. Advanced students may have developed a critical faculty, but how is an undergraduate to judge between the rival theories on, say, protein synthesis or the significance of transpiration? The student, faced with a brief statement of the experimental data and the conclusions drawn by different workers, may well feel himself lost in a morass.

A final criticism concerns the inclusion of statements which can only be attributed to carelessness in proof-reading. Thus on p. 133 we meet the surprising statement that

"in mesophytes, they (the stomata) are almost entirely confined to the lower surface of the leaf." Again, on p. 351, we find "in the leaf, the stomata open in darkness to get rid of excess of carbon dioxide", and on p. 449 the vernalization temperature for winter wheat is given as -2° C. and in the next paragraph we read "Thus winter cereals must not be treated at a higher temperature than 2° to 3° C., and not below 0° C.", and later, on the same page, "An important practical point is that the seed *should not* be immediately sown after treatment" (italics ours) when in fact redrying before sowing is a disadvantage. Ordinary misprints are unfortunately also numerous, especially in the spelling of Latin names.

Parts I and II, which deal with the individual functions, the "classical physiology", of plants, are much better than Part III on growth, reproduction and irritability. The exposition of theories of respiration, and the chapters on osmotic pressure and permeability are particularly well done. As an historical approach to the subject, especially for advanced students, the work has real value and it will undoubtedly find a large public.

R. H. STOUGHTON.

Fundamentals of Biochemistry. By C. L. A. SCHMIDT and F. W. ALLEN. Pp. 388. London: McGraw-Hill Publishing Co., Ltd. 1938. 18*s*.

Of students' text-books of practical biochemistry the two in the English language which are best known are almost certainly "Cole"—a favourite in Great Britain—and "Hawk" which occupies a very similar position in the United States. Both have undergone familiar evolution in the course of the nine (Cole), or eleven (Hawk) editions which have so far appeared. They have started as rather thin, almost entirely "practical" volumes of laboratory experiments, and from edition to edition have steadily increased in size as the amount of text required as background and explanation of the strictly experimental sections has of necessity increased.

The present students' text-book by Schmidt & Allen of the University of California belongs to the general category of the evolved "Cole" or "Hawk". More than that, it belongs to the same high class, which is no mean commendation, and, like the modern editions of these books, combines a clear and straightforward text with a well-digested course of laboratory experiments. Since it is a first edition it has an added freshness of outlook, and is able to concentrate on those sections of biochemistry which are at present the growing points.

From the student's standpoint the general arrangement of the book is most useful. Key papers with which the student should become familiar, in the research literature in each field, are referred to early in most of the chapters. Then a synopsis follows of the essentials of the particular aspect of biochemistry with which the chapter deals, and finally a number, not too many, of experiments on modern lines are described, to be carried out by the individual student or by a small group of students. Of the experiments suggested, several of which are new to any student text-book, some gain force and training value from the fact that they may be carried out, without danger, by the student on himself.

The subjects of chapters include neutrality regulation in the body, enzymes, mineral metabolism, vitamins, endocrines, lipids, bile, carbohydrates, amino acids and proteins, urine and blood analysis, and energy exchange. A few illustrative numerical problems are stated and solved in an appendix. Here it might have been better to have removed the solutions (which are now given in close juxtaposition to the problems) to another part of the appendix. Apart from this minor criticism the reviewer has nothing but praise for a sound, well-arranged text-book of practical biochemistry, a book with flavour and character, and one particularly suited to giving the junior and intermediate student of biochemistry a sound training in laboratory methods.

H. D. KAY.

The Observer's Book of Trees and Shrubs of the British Isles. By W. J. STOKOE. Pp. 240, with 177 illustrations, 16 in colour. London and New York: Frederick Warne and Co., Ltd., 1938. 2*s.* 6*d.*

Descriptions are given of all the British trees, and of several introduced trees commonly grown in British woods and gardens: 106 species are described. The descriptions are accurate and are supplemented by illustrations of the whole tree, the trunk, and in most cases, of the leaves, flowers and fruit. The botanical name and family of each species are given and, with the exception of the misspelling on three different pages of "*araucana*", there are very few misprints. The book is of a convenient size for the pocket and the excellent descriptions and illustrations should enable any interested observer to identify correctly the trees usually grown in this country.

A. G. ERITH.

Mother Earth. By G. W. ROBINSON. Pp. viii + 202, with 6 figures, 2 maps and 1 plate. London: Thomas Murby and Co. 1937. 5*s.* 6*d.*

Prof. Robinson of Bangor is well known as an authority on "soil science" or, as he regards this term as a barbarism, on pedology. His previous volume, *Soils, their Origin, Constitution, and Classification,* of which a second edition was published in 1937, is a standard treatise more suitable for pedologists and agricultural chemists than for workers in other fields or general readers but, as Prof. Robinson believes that "the general reader, perhaps even the farmer and the landowner" ought to be interested in the soil, he has written the present book for their instruction and delectation. In order to express his ideas in a more intimate and direct way than by formal writing he has cast his thoughts into the form of "Letters on Soil" addressed to Prof. Stapledon. He says: "The letters I am about to write will form no systematic course of instruction. They will not form even an elementary text-book of pedology. They are to be considered rather as a series of essays, or even meditations, on topics relating to the soil." "I believe that the story of the soil is as interesting as that of any other part of the great Universe, even though its transactions are fulfilled beneath our feet." And so, in seventeen letters, he discourses on the natural philosophy of the soil, and has produced a book that will do more than all the text-books to interest people in the soil for its own sake, and lead them to think rightly on matters which touch their daily life so intimately.

Commencing with soil materials and structure the author passes to the soil profile and some typical soils, various physical factors, and manures and fertilizers, and shows how all these relate to soil fertility and crop growth. A discussion of soil surveys leads to a consideration of the natural vegetation of the country, agricultural soils, waste lands, and soil destruction and conservation. In a concluding letter the author presents some more personal reflexions on the relation of science to agriculture and to rural policy.

This synopsis gives no idea of the wide scope and thoughtful quality of the book or of the matured knowledge and wisdom it contains. It is finely written and it is a delight to read. At the same time it presents an interesting and beautifully clear picture of achievements, aims and viewpoints in pedology.

WILLIAM B. BRIERLEY.

Genetics and the Origin of Species. By T. DOBZHANSKY. Pp. xvi + 364. New York: Columbia University Press. (London: Oxford University Press.) 1937. 18*s.*

Genetics has developed so rapidly and centrifugally and, in many of its aspects, has become so abstruse, that even the geneticist finds difficulty in breasting the flood. And yet, genetics is so significant for all immediate problems of biology, and has such

far-reaching implications in biological theory, that no biologist dare neglect its advance. For immediate purposes one can pick out occasional genetic researches and attempt to correlate their data with one's own. But there is no intellectual satisfaction in this course, and its superficiality and empiricism may lead to grievous error. One needs to see genetics in its biological setting, in perspective against the general background of the study of living things: one needs to appreciate its relationships and trends, its impacts on cognate or apparently diverse issues, its significance for immediately practical or more remote problems of biology, and its implications in biological theory. The practical and immediate application of genetic knowledge and viewpoints in all aspects of plant culture is obvious, but equally is it obvious that genetic theory and data have direct bearing on biological philosophy, on, for example, the theory of evolution. Yet, genetics is an experimental study of the laboratory and breeding pen, with immediate results and empirical viewpoints, whilst evolution is an observational study of the museum and field, with long scale results and philosophic viewpoints. What is the relation between cytogenetics and natural history; between for example, the immediate micro-evolutionary phenomena of laboratory cultures of *Drosophila* and the macro-evolutionary phenomena of elephant phylogeny in geological time? Can the laboratory methods and phenomena be put to field test or correlated with phenomena in Nature? For any discussion of such problems most of us are dependent upon the more general writings of natural historians or geneticists, and we can only hope that our guides are reliable. Prof. Dobzhansky seems to be equally at home in both fields and a reading of his book gives one the impression that his guidance is trustworthy.

He circumscribes his task as follows: "The present book is devoted to a discussion of the mechanisms of species formation in terms of the facts and theories of genetics... and to see how much of evolution in general can be adequately understood on this basis."

In a first chapter on "Organic Diversity" the author clears the ground and defines three levels of the evolution process: evolutionary statics, or the forces bringing about changes in the genetic composition of populations; evolutionary dynamics, or the interactions of these forces in race and species formation and disintegration; and, thirdly, the realm of fixation of the diversity already attained on the preceding two levels. Evolutionary statics is dealt with in Chs. II, III and IV, devoted, respectively, to gene mutation, mutation as a basis for racial and specific differences, and chromosomal changes. These pages contain, in short compass and readable language, the most critical appraisal of the wider evolutionary relationships of these problems I know.

From this survey the author concludes that gene mutations and numerical chromosome changes are the principal sources of variation. Hereditary variation, thus produced and thrust into a population, enters into the field of action of factors on a different level from those producing variation. These are selection, manner of breeding, and environmental relationships, factors belonging to the realm of the physiology of populations, and concerned with evolutionary dynamics. In Chs. V and VI, therefore, the different agents influencing the fate of the genetic diversity in natural populations are analysed, Ch. VI on "Selection" in particular being a beautifully clear and balanced exposition of this problem.

The next three chapters concern, in general, the third evolutionary level, the fixation of diversity. Ch. VII is an excellent discussion of polyploidy, or the cataclysmic origin of species, the relative prevalence of which in plants and scarcity in animals constitutes the greatest known difference between the evolutionary patterns in the two kingdoms. Ch. VIII is a clear and convincing discussion of the geographical and physiological isolating mechanisms which prevent the interbreeding of groups of individuals, while Ch. IX considers hybrid sterility.

Ch. X on "Species as Natural Units" will, to many readers, be the most interesting but least convincing in the book, and one could wish that the author had allowed himself more scope for the elaboration of his views. Throughout the work he emphasizes the dynamic nature of the species concept, "Species is a stage in a process, not a static unit," and he now defines the species as "That stage of evolutionary

process at which the once actually or potentially interbreeding array of forms becomes segregated in two or more separate arrays which are physiologically incapable of breeding." Such a definition, obviously, is more suitable for animals than for plants and, further, it raises immediately the question of asexual and apogamic organisms. Of these the author says "As in cross-fertilizing organisms, the biotypes in the asexual ones are clustered...the clusters are arranged in a hierarchical order...the different clusters may, then, be designated some as species, others as sub-genera, still others as genera, etc. Which one of these ranks is ascribed to a given cluster is, however, decided by considerations of convenience, and the decision is in this sense purely arbitrary. In other words, the species as a category which is more fixed, and therefore less arbitrary than the rest, is lacking in asexual and obligatorily self-fertilizing organisms. All the criteria of species distinction utterly break down in such forms." Some botanists may find this conclusion rather startling.

Prof. Dobzhansky has written a most thoughtful and stimulating book, and he has a capacity of making difficult things clear without falsifying them by over-simplification, and of putting his ideas concisely and interestingly in simple language. For geneticists the book will be valuable as a clarification of ideas and viewpoints whilst, for those of us who are just ordinary botanists or zoologists, it will be invaluable as enabling us to visualize our science in a wider and deeper perspective. The book contains a useful bibliography which, however, shows surprising omissions.

WILLIAM B. BRIERLEY.

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COMPARATIVE EFFECTS OF COBALT, NICKEL AND COPPER ON PLANT GROWTH

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(With Plates XXIII–XXV and 4 Text-figures)

A. INTRODUCTION

A NOTEWORTHY development of modern physiology has been the recognition of the part played by elements, other than those generally accepted as nutritive, in the economy of the plant. The work has developed along two apparently contradictory lines, in connexion with the toxic effect and the beneficial action of the elements, as in some proved cases the same element may function either way according to circumstances. This contradiction is only apparent, since it is generally accepted that most, if not all, elements, nutritive or otherwise, are poisonous if present in excess, though the limit varies widely with different elements. Attention is now frequently concentrated on the "trace" or "minor" elements, which are often found in very small amounts in soils and plant tissues, and which in some cases are proving of great importance in plant development. Minute traces of boron and manganese are recognized as being essential for growth, and the possibility exists that other elements,

especially copper (Allison *et al.* 1927), may be equally necessary in certain cases. The quantities involved in this way are so small that their investigation needs special technique on account of the difficulty in eliminating unwanted traces of elements from the nutrient substratum. Occasionally, as happened with boron, a fortunate chance gives a "pointer" in the direction of profitable investigation but, more usually, it is necessary to narrow the investigation down from the toxic aspect to the possible beneficial function.

The available information as to the comparative effect of trace elements on plant life is scanty, except in a few cases. While very small amounts may be essential, or may benefit growth, larger quantities may exercise a toxic action which is often obscure, and difficult to recognize if the result is a depression in growth without visible signs of poisoning. The present investigation concerns the second of these possibilities. Cobalt and nickel are of general occurrence in soils, and have been credited with stimulating crops (Nakamura, 1904–5), while recently cobalt has attracted attention on account of its relation to certain animal diseases on soils in which it is deficient. Copper has been included in the work to provide a basis of comparison with an element of considerable economic importance on which more information is available.

B. Historical

The soil content of cobalt and nickel is extremely variable. Kidson (1937) reports a range of 0·3–380 p.p.m. of cobalt in New Zealand soil, the latter figure being outstandingly high. Bertrand & Mokragnatz (1922*a*) found 2·8 mg. of cobalt and 13·6 mg./kg. of nickel in a very fertile arable soil from Pantchevo, near Belgrade, and later (1925*a*) recorded a range of some tenths mg. to 11·7 mg. for cobalt and 5–38·6 mg. for nickel in arable soils from various parts of Europe. Cobalt always seems to be the less in quantity, the Co/Ni ratio varying in their tests from 1/2 to 1/8. High soil content of nickel, and possibly cobalt, may cause damage to crops, as Robinson *et al.* (1935) found that infertile soils derived from serpentine had physical properties that were favourable to plant growth, but contained large quantities of nickel and chromium, and, in one case, of cobalt. Hosking (1936) also determined the cobalt, nickel and copper content of various soils, giving details for both calcareous and non-calcareous types.

The recognition of cobalt as a plant constituent dates back nearly a century, when Legrip (1841) found it in *Lathyrus odoratus*. Forchhammer (1855) then discovered cobalt and probably nickel in the ash of oakwood,

and also stated that the nickel present in peat was very probably derived from the breaking down of vegetation. After another fifty years the more general distribution of these elements in the plant kingdom began to be recognized. Vernadsky (1922) found both nickel and cobalt in all the mosses and several of the phanerogams round Kief. McHargue (1925, 1927) made quantitative estimations, showing traces of the elements in *Poa pratensis* and the leaves of soybean; only a trace of cobalt occurred in soybean seeds, but 3·92 p.p.m. of nickel were present. He also made the suggestion that organic colloidal complexes of these elements, amongst others, might exercise important catalytic functions in the metabolic processes of plants and animals. The same hypothesis was put forward by Bertrand & Mokragnatz (1922*b*, 1925*b*, 1930) who determined the presence of cobalt and nickel in many plants, and concluded that these elements are universally distributed among both phanerogams and cryptogams. Both tend to accumulate in the leaves and seeds, but the quantities are very small, being usually about 1 part of nickel to several million parts of the living plant, while the amount of cobalt is from five to ten times less. Similar results as to the relative distribution of nickel and cobalt are quoted by Ramage (1936), who found nickel usually in traces in various spices, herbal drugs and tea, cobalt being present less frequently. Exceptional amounts occur in St Ignatius' beans, with 0·014% of nickel and 0·003% of cobalt. Martini (1930), again, found nickel to be a normal constituent of plant tissues, occurring in species of the most varied nature, and he also suggested the possibility that it is essential to plant life. Bishop & Lawrenz (1932), on the other hand, claim that while they usually found cobalt in New Zealand spinach, chard, Chinese cabbage and turnips, nickel was always absent, a somewhat surprising result in view of the general consensus of contrary opinion.

The literature on the effect of cobalt and nickel on plant growth is not very comprehensive, and largely consists of isolated observations in connexion with tests of other elements. According to Ducloux & Cobanera (1911–12) very dilute solutions of cobalt salts slightly stimulated *Pisum sativum*, but such stimulation was confined almost exclusively to the leaves, while the roots were usually depressed. In some cases cobalt was eventually found in the leaves as a storage product. Some stimulation of flax was observed by Fukutome (1904) when 0·02 g. of cobalt nitrate was added to 8 kg. of soil. Albano (1915) stated that small dressings of nickel improved the growth of *Corchorus capsularis* (jute), but the quantity recommended as beneficial works out at over 1 cwt. per

acre, which would appear to be a very considerable application. Loew (1924), on the contrary, found no stimulation of cereals with 0·01 g. of nickel sulphate per 2·3 kg. of soil, whereas ten times the quantity caused damage, both these figures being much lower than those of Albano.

All these suggestions of stimulation are somewhat vague, but the evidence for toxicity is clearer, but very scanty. Seedlings of olive trees grown in soil were damaged by cobalt or nickel chlorides applied either in the form of solution added to the normal water supply, or as powder sprinkled on the surface of the soil and carried down gradually by watering. Petri (1910) found that the young shoots were checked by cobalt, and also that an accumulation of the toxic substances occurred, especially in the older leaves, in which the chlorophyll was partly destroyed and the laminae partly dried out by the treatment. The harmful effect of water containing cobalt had already been shown by Haselhoff (1895), from 1 to 2 mg./l. cobalt being sufficient to kill maize and beans grown in water cultures.

The same worker (1893) demonstrated the injurious effect of nickel oxide on horse beans and maize, his concentrations ranging from 2·5 to 50 mg./l. Even the smallest dose poisoned the plants, killing the leaves and seriously hindering development. In the light of more recent work, however, 2·5 mg./l. is in reality a fairly large dose, being equivalent to 2·5 p.p.m., a strength which causes trouble with many compounds. With barley, which was germinated and grown under sterile conditions in nutrient solutions, Wolff (1913) tested nickel sulphate against iron sulphate. Whereas 0·4 g./l. of iron sulphate was very favourable to growth, 0·2 g. of nickel sulphate killed the plants, the result being the same when the concentration was lowered to 0·02 g./l. Cotton (1930) working with buckwheat, indicated that nickel was more toxic than iodine. In the stronger concentrations the stems and leaves lost their turgidity and the young plants died within a fortnight, even 5·87 p.p.m. of nickel being fatal to most plants. The survivors were stunted, newly developed leaves being yellow and covered with small brown spots. Still lower concentrations caused spotting and chlorosis of leaves, as well as depressed growth, and in some cases chlorosis was shown in the presence of as little as 0·59 p.p.m. of nickel. No evidence of stimulation was obtained with still lower concentrations.

Another aspect of toxicity was investigated by Niethammer (1930), who soaked seeds for certain periods in various concentrations of nickel and cyanogen compounds previous to germination. He found that the effect of nickel salts on different species depends upon whether the

solutions can penetrate to the embryo. Wheat grains are penetrated, in spite of their semipermeable membrane, and poisoning occurs. Onions are much less susceptible to parallel strengths, as their seed coats offer a great resistance to penetration. Thiocyanates also penetrate wheat grains, but are less poisonous than nickel salts, and with some strengths stimulation occurs. With older wheat grains, whose vitality has become lessened, nickel salts apparently fail to poison, and may even stimulate.

Results obtained by various workers indicate that compounds of cobalt, nickel and copper are less toxic to the lower than to the higher plants, and more definite evidence of stimulation by low concentrations is available (Ono, 1900; Richards, 1897). Manoilow (1907) found that nickel salts were considerably less poisonous to micro-organisms than those of copper or other metals. On the other hand, Clark (1899) claimed that both nickel and cobalt were more toxic to filamentous fungi than either copper or zinc, though the toxic strengths were considerably above those damaging the higher plants. With *Aspergillus niger* the poisonous action of nickel and cobalt varies considerably, as Mokragnatz (1931) found that though the harmful action of cobalt begins at a strength at which nickel is innocuous, a dose which completely checks the formation of mycelia is lower for nickel than cobalt.

At the present time the importance of cobalt for animal nutrition in certain areas is being so much emphasized that a brief review of the position is given here. The presence of cobalt and nickel in plants and animals was mentioned in a historical account given by Chevalier & Cottereau (1849), but little notice was taken till Bertrand & Mâcheboeuf (1925*a*, 1925*b*, 1926*b*) published a series of analyses showing the distribution of the two elements in various animal organs, both usually being present together. The liver and pancreas of man and the higher animals are the richest organs, nickel being more abundant than cobalt, and marine molluscs are also very rich in nickel. These findings were corroborated by later workers (Dutoit & Zbinden, 1929, 1930; Fox & Ramage, 1931), and nickel was reported by Martini (1929) to be a normal constituent of the mineral salts in bones. Bertrand (and Mâcheboeuf) (1926, 1926*a*) suggested an association of the activity of insulin with the presence of its content of nickel and cobalt, hinting at the possibility of curing certain forms of diabetes by the introduction of small quantities of these elements into the general circulation. Very small traces of nickel and cobalt appeared to be beneficial to white mice (Bertrand & Nakamura, 1927), but in larger amounts both elements come high in the scale of toxicity, the metabolism of rats (Bertrand & Nakamura, 1927; Stare & Elvehjem,

1933; Waltner & Waltner, 1929) and guinea-pigs (Bertrand & Serbescu, 1931) being severely affected, often resulting in death.

During the last few years special attention has been drawn to cobalt in relation to certain deficiency diseases in Australia and New Zealand. Morton Mains disease (Dixon, 1936, 1937*a*; Wunsh, 1937), coast sickness (Lines, 1935), bush sickness (Grimmett & Shorland, 1935), and enzootic marasmus (Underwood & Filmer, 1935) are all forms of sheep disease which have been treated successfully by the administration of small amounts of cobalt, either in the food or by means of drenches. Various investigators, especially Askew *et al.*, have shown, by analyses of soils and pastures, that the incidence of these diseases is associated with local cobalt deficiency, and active experimental work is still in progress (Askew & Dixon, 1936, 1937*a*, 1937*b*, 1937*c*; Askew & Maunsell, 1937; Bell, 1937). Feeding on pastures top-dressed with cobalt salts, drenching the animals with solutions of various types containing small amounts of cobalt, and the provision of cobaltized salt licks, have all proved to be effective methods of combating these diseases. Dixon (1937*b*) has also shown that small quantities of nickel added to a cobalt drench gives better results than cobalt alone. In this country Patterson (1937) states that sheep disease with similar symptoms occurs at Dartmoor on moorland soils with a cobalt content of 3·9 p.p.m., whereas the soils supporting healthy sheep have 16·7 p.p.m.

C. Experimental work

The object of the experiments was to determine the toxicity of nickel and cobalt in view of their widespread distribution in soil. Parallel tests were made with copper to provide a comparison with an element whose action on plant growth is relatively well known.

In considering the effect of various elements on plant growth one of the chief difficulties is the great variation in the nomenclature of the various solutions used, which renders comparison impossible without some form of reduction to a common basis. Every such basis can be criticized for some reason, but perhaps the one least open to objection is that in which the solution of standard strength contains the atomic weight of the element concerned in mg./l. This renders it possible to make direct comparisons between equivalent amounts of the element, regardless of the chemical combination in which it is presented. For the sake of convenience the symbol E has been adopted throughout this work for such a standard solution, so that E = a solution containing the atomic weight of any particular element in mg./l.

With compounds containing one atom of the test element per molecule E therefore represents an $M/1000$ solution, but in cases where two or more atoms are present the equivalent is $M/1000$ divided by the number of such atoms.

Tests were carried out in water cultures using a range of concentrations from $E/4096$ to E, each concentration being two or four times that of the preceding one. The usual Rothamsted nutrient solution was used, with pH 5 for barley and pH 6·2 for broad beans, and the experiments extended over two growing seasons, arranged as follows:

		Barley	Broad beans
1935			
Nickel sulphate, Cobalt chloride, Copper sulphate	In solution	6 March	16 July
	Harvested	18 and 30 July	12 October
1936			
Nickel chloride, Cobalt sulphate, Cupric chloride	In solution	5 March	23 July
	Harvested	24–27 July	23 October

The growing periods for each crop in the two years were very similar in length. In 1935 most of the barley was cut on 18 July, but some plants that were still very immature at that time were left until 30 July before harvesting. In 1936 the whole crop was cut at once, since the previous year's experience had shown that little was gained by delay.

Detailed description of the individual experiments is unnecessary. The control plants and those with concentrations too low to be toxic grew normally, producing good ripe ears in barley, and strong healthy growth in broad beans. The response to toxicity, however, varied with the element and with the species, and some variation also occurred between the compounds in which the elements were presented.

(a) *Barley*

The general trend of response to the action of sulphate and chloride showed certain similarities for each of the three elements, but varied slightly between the compounds (Table I).

(1) *Sulphates.*

With the sulphates, growth was usually much the same in the control solutions and some of the weakest concentrations, after which a marked toxic action became manifest, and the growth curves dropped very suddenly to a point where very little development occurred before the plant died (Text-fig. 1). With nickel sulphate there was a very slight significant fall in the total dry weight between $E/1024$ and $E/64$, the depression being confined to the shoot, not occurring in either root or

Table I. *Dry weights of barley. Grown February–July* 1935, 1936

(Means of 5 plants, in g.)

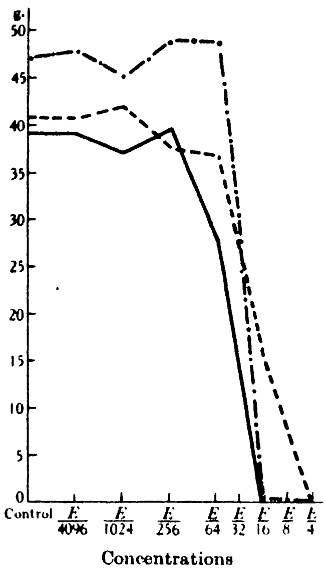
Concentration	Shoot	Root	Grain	Total	Shoot	Root	Grain	Total
	Cobalt							
	Sulphate (1936)				Chloride (1935)			
Control	26·80	4·50	15·83	47·13	25·13	4·28	14·73	44·14
E/4096	27·05	4·13	16·61	47·79	25·75	4·28	15·42	45·45
E/1024	25·54	4·11	15·39	45·04	23·93	3·50	13·77	41·20
E/256	27·27	4·87	16·77	48·91	24·15	4·18	14·71	43·04
E/64	28·37	4·31	15·60	48·68	20·62	3·99	13·04	37·65
E/32	20·03	2·23	5·60	27·86	*			
E/16	0·31	0·09	—	0·40	7·01	1·02	1·13	9·16
E/8	0·12	0·02	—	0·14				
E/4	0·06	0·01	—	0·07	0·09	0·02	—	0·11
E					—	—	—	—
	Nickel							
	Sulphate (1935)				Chloride (1936)			
Control	23·30	3·37	14·32	40·99	26·55	4·31	16·76	47·62
E/4096	23·84	3·73	13·23	40·80				
E/1024	23·92	3·85	14·15	41·92	27·22	4·16	17·10	48·48
E/256	21·19	3·36	12·92	37·47	25·48	3·81	15·88	45·17
E/64	20·18	3·36	13·23	36·77	23·36	3·29	14·25	40·90
E/32					22·59	2·60	11·95	37·14
E/16	9·80	1·70	3·53	15·03	8·99	1·13	0·77	10·89
E/8					0·07	0·02	—	0·09
E/4	0·04	0·01	—	0·05	0·05	0·01	—	0·06
E	—	—	—	—				
	Copper							
	Sulphate (1935)				Chloride (1936)			
Control	21·73	3·93	13·44	39·10	25·31	4·39	16·40	46·10
E/4096	22·24	3·66	13·19	39·09				
E/1024	21·40	3·41	12·29	37·10	24·04	4·12	15·17	43·33
E/256	22·14	3·88	13·44	39·46	21·73	3·00	12·45	37·18
E/64	16·67	2·68	8·29	27·64	21·71	2·93	9·22	33·86
E/32					10·08	1·44	2·15	13·68
E/16	0·08	0·03	—	0·11	0·75	0·28	—	1·03
E/8					0·14	0·05	—	0·19
E/4	—	—	—	—	0·09	0·03	—	0·12
E	—	—	—	—				

* A gap in the table indicates that no experiment was carried out at that concentration, whereas a dash shows that plants were put in nutrient solution but failed to develop. This applies to all tables.

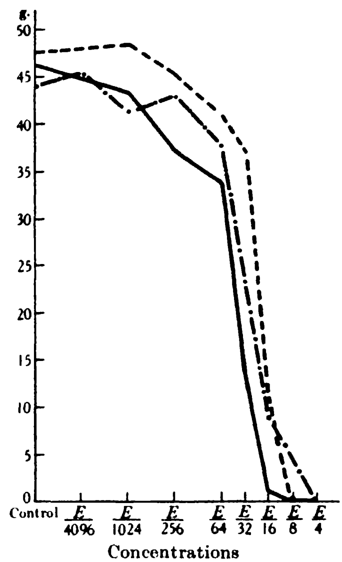
grain. With nickel and cobalt the abrupt decrease in weight set in above the *E*/64 limit, but the toxicity of cobalt was the greater, and *E*/16 practically checked any development. At this strength the plants with nickel sulphate still gave an average dry weight of 15 g., but were very variable, the individuals ranging from 2·0 to 25·68 g. in weight, while at *E*/4 the seedlings died without developing at all. (Pl. XXIII, fig. 2.) Copper sulphate proved to be more poisonous than either of the others,

as the abrupt fall set in between $E/256$ and $E/64$, and practically no growth occurred at $E/16$ (Pl. XXIII, fig. 3).

The greater toxicity of copper showed at an early stage, before the plants had been in the solutions for a week. Checking of root growth was manifest at low concentrations which allowed perfect growth with nickel or cobalt, and the trouble became accentuated as time went on. With nickel sulphate white lesions appeared on the leaves at an early date with $E/16$ and stronger solutions, the roots also being checked, and shortening



Text-fig. 1. Total dry weights of barley grown with varying concentrations of sulphates. —•—•— cobalt; ----- nickel; ——— copper.



Text-fig. 2. Total dry weights of barley grown with varying concentrations of chlorides. —•—•— cobalt; ----- nickel; ——— copper.

of roots appeared later with $E/64$, but did not become very marked. With cobalt sulphate a tendency to checked root growth was soon shown in solutions as dilute as $E/64$ or even $E/256$. The leaves were not injured and eventually the roots grew normally. In higher concentrations the toxic effect was again first shown in the roots, the leaves becoming affected somewhat later.

The toxic action of the three elements was more marked in the grain than in either the shoot or root. With the weakest strengths no harm was done, but with the first sudden drop in dry weight the grain was so depressed as

to lower considerably the proportion between the dry weights of the grain and the whole plant (Table II). With the higher concentrations tested no grain at all was produced in association with the very small shoots and roots.

Table II. *Barley. % of grain in whole plant*

Concentration	Sulphate			Chloride		
	Cobalt	Nickel	Copper	Cobalt	Nickel	Copper
Control	31·89	34·94	34·37	33·37	35·20	35·56
E/4096	34·76	32·42	33·75	33·93		
E/1024	33·80	33·76	33·13	33·42	35·28	36·25
E/256	34·29	34·49	34·05	34·17	35·16	33·49
E/64	32·86	35·98	29·98	36·63	34·85	27·25
E/32	20·10				32·17	15·75
E/16	—	23·50	—	12·30	7·09	—
E/8	—				—	—
E/4	—	—	—	—	—	—
E		—	—	—		

With the concentrations of sulphates that caused marked depression toxic symptoms appeared from the outset, shortened roots being well marked within five days, often accompanied by lesions on the leaves or abnormal, stiff shoot development. In every case this damage persisted and became intensified as time went on. With the borderline concentrations in which the plants were ultimately as good as the controls, as judged by the dry weights, temporary symptoms of toxicity occurred at some stage. With nickel and copper slight symptoms appeared about a fortnight from the start, lasting only a few days with nickel, but persisting for 8 weeks with copper, finally disappearing without doing any permanent damage. With the parallel strength of cobalt 4 weeks elapsed without any determinate signs of toxicity, and then the plant became slightly chlorotic for about a fortnight, soon recovering and producing normal plants.

(2) *Chlorides.*

The range of low concentrations which were innocuous in the case of the sulphates exhibited a certain degree of toxicity when the chlorides were used (Table I). With copper chloride there was a steady significant fall from the control to *E*/64, with nickel chloride from *E*/1024 to *E*/32, whereas, with cobalt chloride, the slight drop was only significant from *E*/256 to *E*/64. (Text-fig. 2). Beyond this range the sudden depression occurred, just as with the sulphates, very little or no growth being made in the highest concentrations.

The visible signs of toxicity were slow in appearing with cobalt chloride, as lesions on the leaves and checking of root growth were not

exhibited so early as with the other elements. Later on, concentrations which at first appeared harmless began to act, and the characteristic marks of poisoning appeared. With concentrations at which marked toxicity first appeared great variation occurred between the individuals with the same treatment, that with the chloride of cobalt and copper being more striking than in the case of the sulphates (Table III, Pl. XXIII, figs. 1, 3).

Table III. *Range of variation between the dry weights of individuals at critical concentrations, in g.*

	Sulphate		Chloride	
	Concentration	Range	Concentration	Range
Cobalt	$E/32$	20·2–30·9	$E/16$	4·3–14·7
Nickel	$E/16$	2·1–25·6	$E/16$	1·6–17·7
Copper	$E/64$	15·2–36·4	$E/32$	6·1–23·7

The critical point beyond which the major depression occurred was much the same with both compounds, but varied with the elements. With cobalt it was $E/64$, and with nickel $E/64$ for sulphate, and $E/32$ for chloride. No test was made with $E/32$ nickel sulphate, and it is possible that this might have been the true critical point for this compound. Copper was the only one in which a marked dissimilarity in behaviour occurred, as the strong depression set in beyond $E/256$ with the sulphate, and $E/64$ with the chloride. Even here, however, the discrepancy was not very marked, owing to the considerable preliminary fall in dry weight with the lower concentrations of copper chloride.

The different effect of the various elements in strong concentrations is well seen by comparing Pl. XXIV, figs. 1–4, all taken on the same day when the plants were 15 weeks old, and the control plants were finely developed and had already produced ears. Even with $E/4$ the different action was manifest, the short bunchy root development with copper being specially marked. This "bunchiness" is caused by the abrupt checking in the growth of the first formed roots, succeeded by the production of a number of subsidiary roots which, in their turn, are checked at about the same stage of development. If the toxic agent is sufficiently strong this bunchy root may persist throughout the life of the plant. With somewhat weaker concentrations, however, the plant eventually becomes strong enough to develop more freely, and the later formed roots begin to elongate in the nutrient solution. In some cases the ultimate root produced may be almost normal in type, showing little signs of the preliminary bunching of the roots. This type of growth is characteristic with many poisonous substances, including copper.

Nickel and cobalt do not come into this category, as even with the strongest concentration the roots were very thin and elongated, the toxic action being manifested in the general feebleness of growth. With decreasing concentration the nickel plants improved rapidly, till at $E/32$ they were too big for comparison on the same photographic plate as the others. The general development with cobalt was similar, but the improvement was less rapid, and at $E/32$ growth was still below normal, indicating the greater toxicity of cobalt compared with nickel. The plants with copper retained their characteristic bunchy roots and depressed growth to $E/32$ and beyond, the improvement in the shoot setting in first.

Nickel and copper chlorides behaved like the sulphates, in that definite toxic symptoms appeared at the outset in the lowest concentrations which ultimately caused great depression, the trouble becoming accentuated with time. With cobalt, toxicity at the early stage was much less definite, 2 or 3 weeks elapsing before it became marked, but the ultimate poisoning was as severe as with nickel. With some of the weaker concentrations of all three chlorides toxic symptoms set in from two to four weeks from the beginning of growth, persisting but not becoming acute, and resulting in the gradual depression of dry weight already discussed.

Comparison of the growth curves shows that, under the conditions of the experiment, the order of decreasing toxicity of the three elements is copper, cobalt and nickel, with both the compounds tested (Text-figs. 1, 2). The extra toxicity of copper persists to a weaker concentration than with either nickel or cobalt. On the other hand, the greater toxicity of cobalt than nickel sulphate is shown most clearly in the higher concentrations, as $E/16$. The true effect of the higher concentration of cobalt chloride is uncertain, as $E/8$ was unfortunately omitted from the test, but judging by the usual slope of the curves it seems probable that very little growth would have occurred at this concentration. The extra toxicity of cobalt is again indicated by the marked depression in growth setting in at a weaker concentration than with nickel chloride. The differences in the weights of the various controls are due partly to the better growth in 1936 than in 1935, and partly to positional effect within the glasshouse. Each individual test, however, was concentrated and randomized, so that comparisons of the effects of treatment are valid.

(*b*) *Broad beans* (Vicia faba)

The tests with broad beans resembled those with barley, except that the weakest concentrations of the toxic substances were omitted, being replaced by more intermediate strengths. Well grown, healthy broad beans always exhibit considerable variations in size, so that no account can be taken of the fluctuations in the dry weights between the control plants and those in the weaker concentrations. The points of sudden

Text-fig. 3. Total dry weights of broad beans grown with varying concentrations of sulphates. —·—·— cobalt; - - - - - nickel; ——— copper.

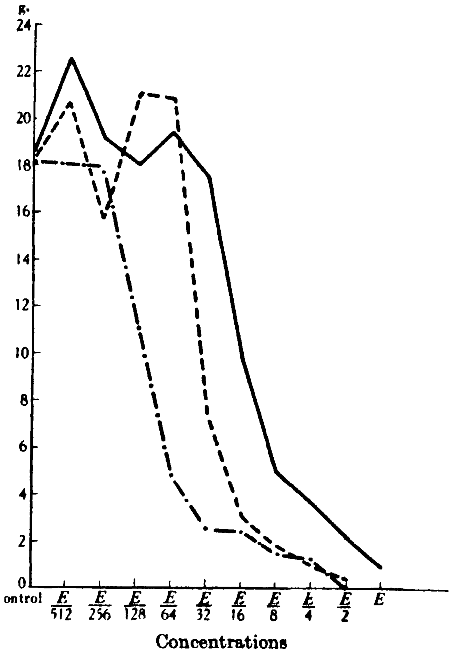
depression, on the other hand, were definitely marked and the course of events with the higher concentrations is clear.

With barley, when once the borderline of toxicity had been passed, the growth curve fell steeply and almost uniformly till the lethal point was reached. With broad beans, the steep fall in the curve again occurred, but as the concentration increased the toxic effect did not increase to correspond, so that more growth was made at the higher strengths than was the case in barley (cf. Text-figs. 1, 3; 2, 4). As will be seen later, this

indicated a greater resistance of broad beans than barley to heavy doses of the poisonous salts tested.

(1) *Sulphates.*

Broad beans varied considerably in the rapidity of their exhibition of damage from poisoning. With nickel sulphate, in less than a fortnight the plants with $E/16$ were definitely poisoned, the roots being stunted and the leaves patchy in colour. As time went on, great depression in



Text-fig 4. Total dry weights of broad beans grown with varying concentrations of chlorides. —•—•— cobalt; - - - - - nickel; ——— copper.

growth was accompanied by a narrowing of the newly formed leaves which were pale and mottled, the roots being short, dark and stubby. By harvest time these effects were intensified, though some plants had produced a few elongated laterals, usually of a thickened type. Weaker concentrations did not at first have any effect, but after about 6 weeks' growth the plants in $E/32$ and $E/64$ showed some thickening and shortening of roots, together with a weakening and paling of the shoots. These effects were temporary, however, as when harvested the plants were

Table IV. *Dry weights of broad bean* (Vicia faba). *Grown July–October* 1935, 1936

(Means of 5 plants, in g.)

Concentration	Shoot	Root	Total	Shoot	Root	Total
	Cobalt					
	Sulphate (1936)			Chloride (1935)		
Control	11·76	3·95	15·71	13·74	4·40	18·14
$E/512$	12·42	3·70	16·12			
$E/256$	11·27	2·89	14·16	13·57	4·32	17·89
$E/128$	3·57	0·81	4·38			
$E/64$	2·08	0·42	2·50	3·74	1·01	4·75
$E/32$	1·53	0·39	1·92	1·91	0·58	2·49
$E/16$	1·06	0·19	1·25	1·86	0·52	2·38
$E/8$	0·88	0·18	1·06	1·18	0·23	1·41
$E/4$	Not separated		0·65	1·08	0·15	1·23
$E/2$	,,	,,	0·41	—	—	—
E				—	—	—
	Nickel					
	Sulphate (1935)			Chloride (1936)		
Control	16·20	4·11	20·31	14·14	4·16	18·30
$E/512$				15·60	5·00	20·60
$E/256$	18·40	5·26	23·66	11·93	3·69	15·62
$E/128$				16·48	4·59	21·07
$E/64$	14·40	5·10	19·50	15·68	5·16	20·84
$E/32$	13·47	4·83	18·30	5·25	2·06	7·31
$E/16$	4·23	1·07	5·30	2·16	0·88	3·04
$E/8$	2·42	0·68	3·10	1·23	0·52	1·75
$E/4$	1·00	0·19	1·19	Not separated		0·90
$E/2$	-	—	—	0·32	0·07	0·39
E	—	—	—			
	Copper					
	Sulphate (1935)			Chloride (1936)		
Control	18·32	5·06	23·38	13·99	4·82	18·47
$E/512$				17·01	5·49	22·50
$E/256$	15·07	4·52	19·59	14·67	4·46	19·13
$E/128$				14·39	3·55	17·94
$E/64$	15·71	5·04	20·75	15·27	4·10	19·37
$E/32$	8·37	2·52	10·89	14·27	3·18	17·45
$E/16$	5·06	1·22	6·28	7·60	2·07	9·67
$E/8$	5·13	1·45	6·58	3·86	1·05	4·91
$E/4$	3·83	1·16	4·99	2·66	0·96	3·62
$E/2$	1·25	0·43	1·68	1·59	0·65	2·23
E	—	—	—	0·50	0·34	0·84

healthy and normal, except that those with $E/32$, just below the border-line of toxicity, were somewhat unusually variable. After the abrupt drop in weight at $E/16$, the toxic effect decreased, and total inhibition of growth did not occur till $E/2$ (Table IV, Text-fig. 3). Copper sulphate, near the critical strength, was much slower in coming into action. With $E/32$, which finally caused marked depression in growth, no ill-effects were obvious for a long time, since after 6 weeks' growth the roots were

Table V. *Heights of broad bean* (Vicia faba)

Mean of 5 plants, in cm.

	Cobalt	Nickel	Copper
		Sulphate	
Control	82·9	80·8	97·3
$E/512$	73·1		
$E/256$	79·6	81·4	82·6
$E/128$	49·4		
$E/64$	38·4	81·6	92·7
$E/32$	28·1	79·3	76·3
$E/16$	24·9	41·3	61·7
$E/8$	19·5	32·4	61·0
$E/4$	11·9	15·0	54·3
$E/2$	7·0	—	33·0
E	—	—	—
		Chloride	
Control	80·1	102·3	104·1
$E/512$		101·4	113·7
$E/256$	74·0	90·0	100·1
$E/128$		91·4	106·0
$E/64$	50·5	89·5	99·3
$E/32$	33·8	42·1	112·5
$E/16$	37·4	31·9	91·4
$E/8$	17·5	25·9	69·6
$E/4$	18·8	8·2	57·5
$E/2$	—	3·5	46·3
E	—	—	4·5

only beginning to become short and bunchy, and the shoot to produce narrow and pale leaves. By harvest time the growth was considerably less reduced than is usual when marked toxicity sets in. After a continued rapid drop to $E/16$, the depression of growth was slower, and the plants were much larger than with similar strengths of nickel sulphate, E being the only concentration which killed the plants outright at the beginning. From $E/8$ to $E/2$ the roots were typical, with short, thick laterals, often curved, bunched together on the main root. The tips of the laterals were often curved, the apices often swollen, while the cortex of the main root was frequently split from the pressure of the mass of rootlets trying to emerge from the stunted root. All these malformed roots were dark brown or black in colour, and were accompanied by tall shoots which showed a marked contrast in size with the corresponding shoots in nickel and cobalt sulphate (Table V).

The poisonous effect of cobalt sulphate was even slower in manifesting itself than that of copper sulphate. Five weeks elapsed before the plants with $E/128$ showed any sign of trouble, and then they were rather more variable than normally and the roots were beginning to thicken. A fortnight later they were still as tall as the controls, but were turning yellow

and some of the apical leaves were withering. Yet, when harvested in October, great depression of growth had occurred, and most of the shoots were much shorter than the controls and were dead or dying, indicating severe poisoning (Pl. XXV, fig. 1). This behaviour affords a good instance of the danger of drawing conclusions with regard to toxicity or stimulation from plants that are too young and immature. The absence of symptoms at an early stage does not necessarily imply inaction, as trouble or benefit may set in at a later stage of growth. In this case, the effect of nickel sulphate was manifest at an early date and persisted throughout whereas, with copper and cobalt sulphate, concentrations that at first appeared innocuous proved later to be strongly toxic.

(2) *Chlorides.*

The gradual depressing action of low concentrations of the chlorides, which occurred with barley, was not evident with broad beans (Text-fig. 4). The dry weight with the strongest concentration that did not cause marked depression was in no case significantly below that of the control (Table IV). With nickel and cobalt the onset of the toxic symptoms was somewhat slow, three or four weeks elapsing before the plants showed the characteristic variability and definite signs of poisoning. At this time $E/32$ nickel chloride had caused much shortening and thickening of the roots, while the leaves were yellowish and mottled. With age the symptoms were intensified and numerous small brown spots appeared on the apical leaves. With stronger concentrations the thickening of the roots was abnormal, but some of the plants just managed to survive at $E/2$ (Pl. XXV, fig. 2).

Copper chloride proved innocuous to $E/32$, but toxic symptoms appeared almost at once with $E/16$ and persisted throughout growth (Pl. XXV, fig. 3). As with copper sulphate, however, the behaviour differed from that with nickel and cobalt, since the depression was less great at the first toxic concentration and continued at much the same rate to the next, in this case $E/8$, after which the associated fall in dry weight decreased considerably. Even at $E/2$, the plants had shoots 46 cm. long, but E had prevented any development of root or shoot. The strengths at which the elements allowed maximum growth were reversed with the two compounds, being $E/64$ for copper sulphate and nickel chloride, but $E/32$ for copper chloride and nickel sulphate. It is not possible, from the data available, to say if this difference is constant or really significant.

Cobalt chloride, like the sulphate, was the most toxic, $E/256$ being the greatest strength which permitted normal growth. At $E/8$ copper salts,

though markedly poisonous, permitted a fairly strong root growth, and even at four times the strength, with $E/2$, the root development with copper was better than with $E/8$ cobalt sulphate, in which practically no development of laterals had occurred (Pl. XXIV, figs. 5, 6). After the delayed appearance of signs of poisoning, the action was rapidly intensified, and the leaves were eventually much blotched and the roots unhealthy. With higher concentrations the bursting of the root cortex by the crowded and suppressed laterals was marked.

D. Discussion

In all work involving the association of toxic compounds and nutrient salts various factors are liable to influence comparisons. In earlier Rothamsted work (Brenchley, 1910, 1927) in which barley was grown with copper sulphate ($CuSO_4 . 5H_2O$) with and without nutrient salts, 1 p.p.m. of the sulphate alone sufficed to check all root growth. In the presence of nutrient salts, on the contrary, almost normal growth was made with this concentration, 1/100,000 being necessary to cause the same amount of damage, ten times as much as in the other test. There is always the possibility that more or less of the toxic substance may enter into insoluble combinations with some of the food salts and so be removed from the solution, thus weakening the concentration. With the nutrient solution used, such insoluble compounds would probably be phosphates. The proportion of potassium phosphate in the food salts is relatively heavy, about 0·5 g./l., whereas the quantity of toxic substance is small, the maximum amount of the poisonous element permitting normal growth in the present experiments ranging from 0·2–2 mg./l., according to circumstances, all greater amounts being extremely poisonous. If precipitation occurred, it might be expected that the overwhelming quantity of potassium phosphate could throw out much more of the toxic compound than was actually present, but the varying behaviour of the two species in identical solutions suggests that such precipitation probably does not occur here. Again, the fact that most "insoluble" substances are really very slightly soluble is of importance and, with the weaker concentrations at least, any phosphatic compound formed might be within the range of such solubility, thus leaving the whole of the poisonous compound in the solution. The degree of solubility would be affected by the *p*H of the nutrient solution.

From the practical and economic point of view, however, the important aspect of toxicity is in relation to plants growing with an adequate food supply, which may be presented normally in soil or

experimentally in nutrient culture. From this point of view the comparisons in the present series of experiments are valid, regardless of what reaction may take place between the poisonous and nutrient salts, since they indicate the trend of events under parallel conditions of plant growth. Jensen (1907) found that in solution cultures the lethal point of nickel nitrate for wheat plants was $n/2000$–$n/400$, but that by the addition of quartz to the solution the lethal limit was raised to $7n/2000$–$6n/4000$, a reduction of toxicity of six to seven times. In soil the toxic effect of poisonous compounds is usually much reduced, owing to adsorption by the solid particles, so that the level of dangerous concentrations may be considerably raised. In the Hill's pot culture experiments at Woburn, Voelcker (1913) found that wheat plants were damaged by copper sulphate supplying 0·05% of copper reckoned on the weight of soil used; copper carbonate proved nearly as harmful, the nitrate was more toxic, whereas the insoluble phosphate could be used in much larger amounts without any injurious effects. These quantities are much larger than those which can safely be used in nutrient solution, showing that much of the toxic substance must have been inactivated by interaction with the soil.

It is generally recognized that all species do not respond to the same poison in the same way, but much of the work on the subject deals only with the early stages of growth and information covering the whole life cycle is comparatively scanty. In these experiments on cobalt, nickel and copper the general trend of response was much the same in all cases. Over a range of low concentrations growth was either similar to that of the controls or showed only a gradual depression. This was followed by a sudden check in development with the succeeding concentration, the point at which this occurred varying with the species and the toxic compound. The ultimate behaviour with greater strengths again varied.

With barley the general order of toxicity was copper > cobalt > nickel, though the difference was not very strongly marked and depended somewhat upon the compound used. With broad beans the greater toxicity of cobalt was outstanding, very little growth being made in comparatively low concentrations at which nickel and copper were inactive. Copper, on the other hand, was the least poisonous and, in strong concentrations, permitted an amount of growth that did not occur either with nickel or cobalt on beans, or with any of the three when applied to barley. The general order of toxicity with beans was cobalt > nickel > copper, the differences being greater than in the case of barley. Scharrer & Schropp (1933) carried out short-term experiments for about a fortnight with a variety of plants in culture solutions, and indicated that at low concen-

trations nickel was not so poisonous as cobalt, but at high concentrations there was little difference in their action. The latter statement, however, is now shown not to hold good consistently when plants are grown to maturity since, in Rothamsted tests, considerable variations in relative toxicity occurred in the higher concentrations. The greater tolerance of barley than beans to cobalt was shown in both sets of experiments. In earlier work by Coupin (1901) wheat seedlings were grown for twelve days in dilute solutions of the poisons dissolved in distilled water without nutrients. He found that, under these conditions, corresponding compounds of nickel and cobalt had very similar toxicities, the nitrates being much less toxic than the chlorides and sulphates. In India, Singh & Prasad (1936) found that wheat grown in Knop's solution exhibited slight toxicity with cobalt chloride at $0{\cdot}0001\,M$ ($=E/10$), the roots at 8 weeks being only 8·6 cm. long, compared with 22·6 cm. in the controls. At maturity the ears with cobalt ripened earlier, but the grain did not set well. This concentration is high compared with that permitting so much growth in barley, but the discrepancy may be due to the great difference in climatic conditions as well as to species response.

The general effect of the various sulphates and chlorides was not consistent, and no specific statement can be made as to which is the more toxic (Table VI). With cobalt the critical strength was identical in both the species tested. With copper the sulphate was more toxic than the chloride in both cases, and also was more poisonous to barley than to broad bean. With nickel, the relative toxicity was reversed both with the compounds and with the species, but as the concentrations were at such close range it is possible that the action was really similar, as in the case of cobalt.

Table VI. *Relative toxicities. Lowest concentrations which did not cause marked depression in growth*

		Barley	Broad bean
Sulphate	Cobalt	$E/64$	$E/256$
	Nickel	$E/64$	$E/32$
	Copper	$E/256$	$E/64$
Chloride	Cobalt	$E/64$	$E/256$
	Nickel	$E/32$	$E/64$
	Copper	$E/64$	$E/32$

The chlorides, acting on barley, provided the only instance in which very low concentrations caused a gradual depression of growth before the characteristic sudden drop. In other cases the same concentrations were inactive, giving plants as good as the controls. With higher strengths the barley died quickly, whereas beans made more growth. This may partly

be due to the different size of the seed, the amount of reserve food contained in the bean enabling the plant to make some growth in the presence of an amount of poison which prevents barley from utilizing inorganic food salts from the nutrient solution after its own small reserves are exhausted. Further work with other species of plants and under different conditions of growth is necessary to settle this point, and to determine the possible economic importance of nickel and cobalt when they occur together in the same soil.

E. SUMMARY

1. An account is given of the present position of our knowledge with regard to the distribution and the physiological importance of nickel and cobalt, in relation to plants and animals.

2. Experiments on barley and broad beans were carried out in water cultures with the sulphates and chlorides of cobalt, nickel and copper.

3. In every case a range of low concentrations did little or no damage, but toxic action occurred abruptly above a concentration which varied with the species and with the compound.

4. With barley, copper was the most poisonous element in either compound, but the differences were not striking. Low concentrations of the sulphate were innocuous, but parallel low strengths of the chloride caused a slight, significant depression in growth.

5. With broad beans, cobalt was much more poisonous than either nickel or copper, particularly with the sulphate. No slight depression with low concentrations of the chloride was noticeable with this species.

6. The morphological response to toxicity varied with the element concerned. Copper, in poisonous strengths, caused shortening and "bunching" of barley roots, whereas nickel and cobalt permitted the growth of elongated roots of a very attenuated nature.

7. The individuality of plant response to poison was frequently shown by the great variation in growth in the borderline concentrations just below those which caused marked depression of growth.

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EXPLANATION OF PLATES XXIII—XXV

PLATE XXIII

Fig. 1. Barley grown in nutrient solution with cobalt chloride. Concentrations: (*a*) control, (*b*) $E/64$, (*c*) $E/16$ (group of three), (*d*) $E/4$.

Fig. 2. Barley grown with nickel sulphate. Concentrations as fig. 1.

Fig. 3. Barley grown with copper sulphate. Concentrations as fig. 1.

PLATE XXIV

Figs. 1–4. Barley grown with nickel chloride, copper chloride and cobalt sulphate, in concentrations $E/32$, $E/16$, $E/8$, $E/4$ respectively. Photographed after 15 weeks' growth.

Fig. 5. Broad bean roots, showing greater toxicity of cobalt compared with copper. Concentrations, cobalt sulphate, (*a*) $E/16$, (*b*) $E/8$.

Fig. 6. Broad bean roots as fig. 5. Concentrations, copper chloride, (*a*) $E/8$, (*b*) $E/4$, (*c*) $E/2$.

PLATE XXV

Fig. 1. Broad beans grown in nutrient solution with cobalt sulphate. Concentrations: (*a*) control, (*b*) $E/512$, (*c*) $E/256$, (*d*) $E/128$, (*e*) $E/64$, (*f*) $E/32$, (*g*) $E/16$, (*h*) $E/8$, (*k*) $E/4$, (*l*) $E/2$.

Fig. 2. Broad beans with nickel chloride. Concentrations as fig. 1.

Fig. 3. Broad beans grown with copper chloride. Concentrations as fig. 1, with addition of (*m*) E.

(*Received* 18 *February* 1938)

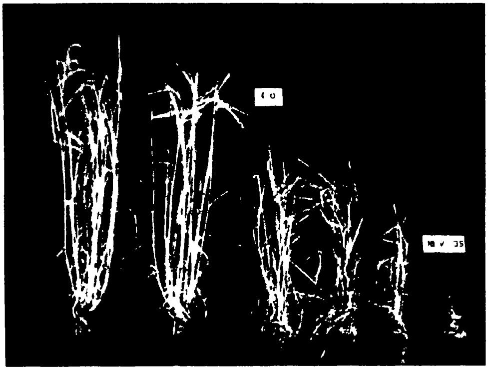
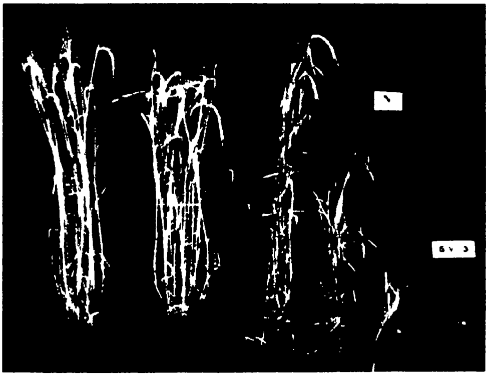


Fig. 1



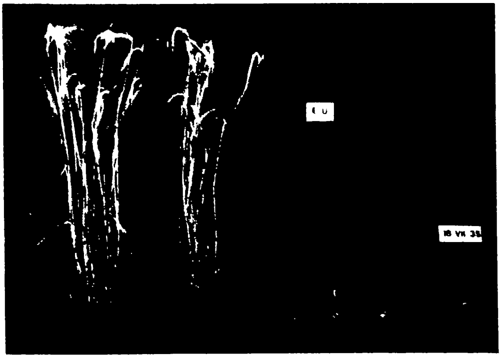
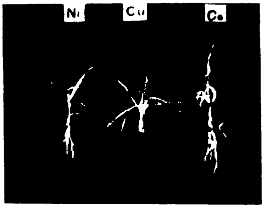


Fig. 3.

Fig. 1.

Fig. 2.



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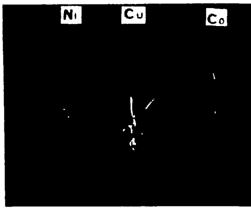

Fig. 4.

Fig. 5.

Fig. 6.

BRENCHLEY.—COMPARATIVE EFFECTS OF COBALT, NICKEL AND COPPER ON PLANT GROWTH (pp. 671–694).

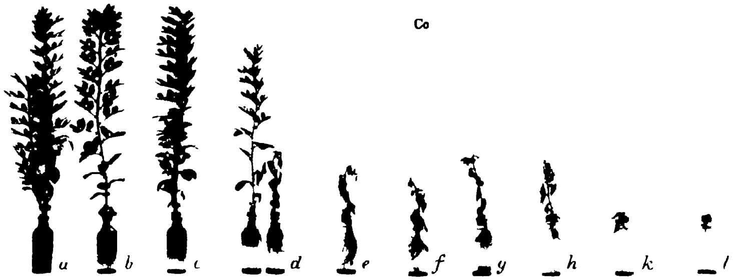

Fig. 1.

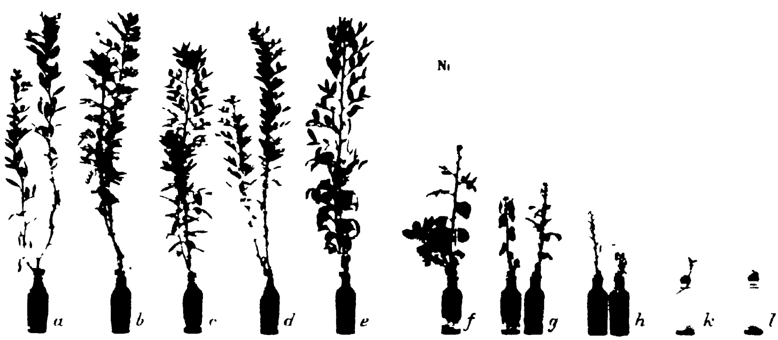

Fig. 2

Fig. 3.

BRENCHLEY. COMPARATIVE EFFECTS OF COBALT, NICKEL AND COPPER ON PLANT GROWTH (pp. 671–694).

CROSS- AND SELF-FERTILITY IN SAINFOIN

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CONTENTS

Introduction

An essential preliminary to any scheme of crop improvement or seed production is to find out to what extent the particular species under consideration is capable of setting seed by self-pollination. This knowledge will determine the breeding methods to be used and the precautions necessary to maintain the purity of stocks. In the case of leguminous herbage plants the problem has been investigated in numerous species. Williams (1931 *a*, *b*, *c*, *d*) has examined species of *Trifolium*, *Medicago* and *Anthyllis* at Aberystwyth and has classified them into three categories: (1) voluntarily self-fertile species, i.e. species which are able to effect self-pollination and fertilization without the aid of external agents; (2) species which are practically incapable of effecting self-pollination without the aid of external agents, but which are fully self-fertile when artificially self-pollinated; and (3) species which are almost completely self-sterile even when artificially self-pollinated.

As regards sainfoin, Fleischmann (1932), in Hungary, investigated seed setting in Persian sainfoin (*Onobrychis viciaefolia* var. *persica* Sir.) and found that racemes set an average of 25·5 seeds when left open, though there was considerable variation in the actual numbers. When the racemes were bagged, one quarter of them set no seed, while the remainder

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set from one to nine, the average number of seeds per raceme being 1·5. An account of the pollination mechanism has been given by Müller (1883). In the present work the relative importance of cross- and self-pollination in the varieties of sainfoin grown in England (*O. sativa* Lam. = *O. viciaefolia* var. *sativa* Lam.) has been examined.

Materials

The work was done at the Agricultural Botanic Garden of the University of Reading. Seeds of the Giant and Common varieties of sainfoin were sown in drills in the spring of 1935. Giant sainfoin flowers in its seeding year and, in subsequent years, flowers two or three times so that two or three cuts can be taken. Common sainfoin does not flower in its seeding year, and in subsequent years flowers only once, providing only one hay cut followed by grazing. The investigations were made in May and June 1936 and 1937. Seeds of giant sainfoin are harvested normally after the second flowering, but these experiments were carried out at the first flowering in order to give a more accurate comparison with common sainfoin which flowers once only.

Pollination

The inflorescence of sainfoin is a dense spike-like indefinite raceme borne on a long axillary peduncle. The flower has a very short pedicel and is subtended by a small subulate bract, longer than the pedicel. The calyx is pubescent and has five long pointed teeth and a short tube. The polypetalous corolla is pink with red veins. The standard is notched at the apex and the limb is slightly turned up. The keel is almost as long as the standard, its claws are short and its limbs transversely truncate. The wings are half-sagittate, very small and completely hidden. The stamens are fused for two-thirds of their length, except the posterior one which is free and shorter than the others. The ovary is ovoid and pubescent, containing a single ovule and is borne on a short gynophore. The style is long and the terminal third is bent up at right angles and projects beyond the anthers. At the apex of the raceme is a tuft of undeveloped flowers. The flowers open from the base of the raceme upwards and a period of ten to twelve days elapses between the opening of the first flower and the withering of the terminal flowers.

In consequence of the small size of the wings, the full weight of a visiting insect is taken by the keel. When this is depressed the stigma and anthers come into contact with the insect's body and, when it is released, the keel returns to its original position. A flower may therefore be tripped

by an insect several times. According to Knuth (1906), the style becomes more erect as anthesis progresses, ultimately projecting 1–1·5 mm. from the keel cleft, thus excluding automatic self-pollination and ensuring cross-pollination.

As the calyx tube is short and the petals completely free, the nectar is easy of access and a variety of insect visitors might be expected. Knuth (1906) lists 52 insects which have been observed, mostly by Müller, to visit sainfoin in Central Europe. This list is made up of 41 Hymenoptera, one Dipteron and ten Lepidoptera. According to Müller's observations (1883), the honey bee is by far the most frequent visitor, paying nine out of every ten visits recorded. At Shinfield the most frequent visitor was the honey bee; other insects observed were humble bees and various Lepidoptera. Darwin (1878) records an occasion when a field of sainfoin was cut and he observed honey bees "driven to desperation" attempting to obtain nectar from red clover in an adjoining field.

NUMBER OF FLOWERS PER RACEME

On the stem of sainfoin, racemes arise in succession from the lower nodes upwards and as many as five or six may be produced. The numbers of flowers in the three lowermost racemes were counted. On 27 May 1937 the lowermost raceme was taken for counting from one stem of each of 50 common sainfoin plants chosen at random. Similarly, 50 racemes of giant sainfoin were taken on 29 May. When the second and third racemes came into flower, 50 each of giant and common sainfoin were removed for counting. There was, therefore, a set of 100 of each of the first three

Table I

No. of flowers per raceme	% frequency	
	Giant	Common
10–19	2·7	0·7
20–29	2·7	4·0
30–39	16·0	10·0
40–49	26·0	17·3
50–59	20·0	18·7
60–69	19·3	20·7
70–79	6·7	16·7
80–89	3·3	6·7
90–99	2·0	4·7
100–109	0·7	0·7
110–119	—	—
120–129	0·7	—
No. of racemes counted	150	150
Mean	52·7	58·9
S.E.	1·42	1·45
Average of giant and common	55·8	

racemes, one half being from giant and one half from common sainfoin. The three sets were not necessarily taken from the same stems or from the same plants.

The counts are given in Table I in which those from the three sets of racemes are combined. Considerable variation was found in the number of flowers per raceme. The number ranged from 15 to 127, but the majority of racemes contained between 30 and 80. The mean number of flowers per raceme was found to be 58·9 in common and 52·7 in giant sainfoin, the difference being significant. Taking the giant and common varieties together, the mean number was 55·8.

The figures were analysed to discover if there was any difference in the number of flowers per raceme at different nodes. Combining the figures for the two varieties, the numbers of flowers were:

1st raceme – $58·4 \pm 2·00$
2nd ,, – $56·3 \pm 1·50$
3rd ,, – $52·7 \pm 1·77$

These figures appear to indicate that the later formed racemes bear fewer flowers than the earlier ones, but the differences, even between the first and third, are not significant.

FRUIT SET

The numbers of fruits per raceme were counted in the same way as the numbers of flowers. The results are given in Table II and show considerable variation in the number of fruits set. The number of fruits per raceme ranged from 1 to 72, but the majority of racemes bore between 10 and ·50. In giant sainfoin the mean number of fruits per raceme was 29·37 and in common sainfoin 31·03, but the difference is not significant. Taking the two varieties together, the mean number of fruits was 30·2.

Table II

No. of fruits per raceme	% frequency	
	Giant	Common
0–9	9·3	1·3
10–19	17·3	14·7
20–29	28·7	35·3
30–39	20·7	30·0
40–49	13·3	9·3
50–59	6·7	4·0
60–69	2·7	4·7
70–79	1·3	0·7
No. of racemes counted	150	150
Mean	29·37	31·03
S.E.	1·245	1·045
Fruit set	55·7 %	52·7 %
Average fruit set of giant and common	54·1 %	

The "fruit set" was obtained by expressing these figures as percentages of the number of flowers per raceme (Table I). In giant sainfoin the fruit set was 55·7% and in common sainfoin 52·7%, there being no significant difference, and the average of the two varieties was 54·1%. Thus little more than half the flowers set seed.

The numbers of fruits set at different nodes were found to be as follows:

	No. of fruits	Fruit set
1st raceme	28·6 ± 1·45	50·0%
2nd ,,	33·9 ± 1·57	60·2%
3rd ,,	28·1 ± 1·08	53·3%

The second raceme had a higher fruit set than the first or third but the difference is not significant.

SELF-FERTILITY

The extent to which sainfoin is capable of setting seed by self-pollination was studied in 1936 and 1937. Thirty-six giant plants and 39 common plants were used. On each plant three racemes with unopened buds were chosen. Two of these were enclosed in wax paper bags plugged with cotton-wool and the third was left as a control. One of the bagged racemes was left untouched until the fruits had set. The other had its bag removed every day while in flower, the open flowers were tripped with a sterile blunt needle and the bag replaced. The only chance of cross-pollination was from wind-borne pollen during the tripping process, but as this took only about a minute, there was little possibility of this happening. When the fruits had set and had developed sufficiently to ensure that they each contained a seed, the bags were removed and the fruits counted (Table III).

When the flowers were tripped 20 racemes set no fruits, and the number of fruits in the remaining 55 racemes varied from 1 to 17. The mean number of fruits set per raceme with tripped flowers was 2·853. When the flowers were left untouched, 53 racemes set no seed and, in the remaining 22 racemes, the number of fruits set varied from one to four. The mean number of fruits set per raceme with untripped flowers was 0·547. The difference between the fruit-sets of tripped and untripped racemes is significant. The controls, which were open to free pollination by bees set 28·767 fruits per raceme. The fertility was calculated by expressing these figures as percentages of 55·8, the mean number of flowers per raceme (Table I). The fertilities were 5·11, 0·98 and 51·55% for tripped, untripped, and control racemes respectively.

That the degree of self-fertility in sainfoin is very low was confirmed by other observations made in 1937. Five 2-year old plants were growing

Table III

No. of fruits per raceme	Frequency: Flowers tripped	Flowers not tripped	Controls
0	20	53	—
1	14	12	—
2	12	4	—
3	7	3	—
4	6	3	—
5	4	—	—
6	2	—	1
7	1	—	—
8	2	—	—
9	2	—	—
10	3	—	1
11	1	—	1
12	—	—	2
13	—	—	2
14	—	—	1
15	—	—	1
16	—	—	2
17	1	—	5
18	—	—	2
19	—	—	2
20–29	—	—	22
30–39	—	—	20
40–49	—	—	9
50–59	—	—	2
60–69	—	—	1
70–79	—	—	1
No. of racemes counted	75	75	75
Mean	2·853	0·547	28·767
S.E.	0·3845	0·1196	1·4480
Fertility	5·11%	0·98%	51·55%

in pots in a greenhouse. The pots were standing together and no attempt was made to protect the flowers against cross-pollination, but no bees were ever observed in the house. These five plants produced 231 racemes, and 217 of these produced no fruits. Of the remainder, ten set one fruit each, two set two fruits each, one set three fruits and one set four fruits, the average for the 231 racemes being 0·09.

The figures were analysed to ascertain if there was any difference between the two varieties or between the two years in which the experiments were carried out.

Taking the results of 1936 and 1937 together, the numbers of fruits per raceme were:

Flowers tripped:	Giant	$2{\cdot}472 \pm 0{\cdot}4992$
	Common	$3{\cdot}205 \pm 0{\cdot}5726$
Flowers untripped:	Giant	$0{\cdot}556 \pm 0{\cdot}1592$
	Common	$0{\cdot}538 \pm 0{\cdot}1771$

These slight differences between giant and common sainfoin are not significant.

Taking the giant and common varieties together, the numbers of fruits per raceme were:

Flowers tripped:	1936	$2{\cdot}961 \pm 0{\cdot}5189$
	1937	$2{\cdot}167 \pm 0{\cdot}5609$
Flowers untripped:	1936	$0{\cdot}628 \pm 0{\cdot}1491$
	1937	$0{\cdot}50 \pm 0{\cdot}2205$

The differences between the two years are not significant.

Seedlings from selfed and crossed seeds

Seeds obtained by self-fertilization in 1936 were sown in the open in 1937 and the seedlings kept under observation. Out of 47 selfed seeds from giant sainfoin only 5 gave established seedlings, and out of 45 selfed common seeds 18 gave established seedlings. The seedlings appeared to be normal morphologically, but in some growth was very slow. Growth was very poor in the five giant seedlings, though one produced flowers. Of the 18 common seedlings growth was recorded as unsatisfactory in five. These observations are not conclusive, but they suggest that Common is more likely than giant sainfoin to give vigorous seedlings from selfed seed.

Seeds from the control racemes were sown for comparison with the selfed seeds. No establishment values were taken but, in comparison with the seedlings from selfed seeds, practically all the established seedlings grew vigorously. Of 31 giant seedlings 25 flowered, and of 34 common seedlings none flowered.

Discussion

The difference between the giant and common varieties in the number of flowers per raceme should be considered in conjunction with differences in the vegetative characters of the seedlings. It has been shown (Thomson, 1938) that the leaves of giant sainfoin seedlings tend to have a greater number of leaflets than the leaves of common sainfoin seedlings. In flower production, however, the position is reversed, common sainfoin having a greater number of flowers per raceme than giant sainfoin.

Out of an average of 55·8 flowers per raceme, little more than one half set seed under normal conditions of open pollination. The number of fruits set per raceme, 30·2, was rather higher than the figure given by Fleischmann (1932) for Persian sainfoin growing in Hungary. The proportion of

flowers setting seed was of the same order as that found for red clover by Williams (1931*a*). There is no evidence to suggest a possible reason for this low fertility. According to Williams (1927) the number of bees may be a limiting factor in the seed production of red clover, and this may also apply to sainfoin, but there appeared to be no shortage of honey bees on the plots.

The maximum self-fertility possible when every stigma is dusted with its own pollen by artificial tripping is 5·11 %. In the absence of tripping agents the fertility is 0·98 %. It appears, therefore, that sainfoin is slightly self-fertile but is usually cross-pollinated. The reduction of fruit-set from 5·11 to 0·98 % when the flowers are not tripped shows that the mechanism described by Knuth (1906) as preventing automatic self-pollination is fairly effective. The number of fruits set per raceme when the flowers were untouched was lower than that found by Fleischmann (1932) in Persian sainfoin but, as he does not state the number of flowers per raceme, it is impossible to say whether Persian sainfoin shows any significant difference in self-fertility. Reference has been made above to Williams's classification of herbage legumes according to their self-fertility. Sainfoin fits into none of his three categories, but is intermediate between the second and third, being almost incapable of pollinating itself but slightly self-fertile when artificially self-pollinated. The self-fertility was very low when the plants were grown under glass and no difference was found between the two seasons 1936 and 1937. This indicates that self-fertility is uniformly low and is not greatly influenced by environmental conditions as in Lucerne (Williams, 1931*c*). Any scheme of improvement in sainfoin must therefore depend on building up families and not on selection of single plants and subsequent self-pollination.

The reduction in vigour of the selfed seedlings is what might be expected, though there were no chlorophyll-deficient seedlings such as occur in red clover (Williams, 1931*a*).

Rees (1928) found that some lots of common sainfoin flowered in the seeding year. Certain regional strains of common sainfoin are intermediate between the giant and the true common variety (Thomson, 1938), but Rees has pointed out one possible reason for seeding year flowering which may be mentioned, namely that the parent common sainfoin plants were fertilized by pollen from neighbouring fields of giant sainfoin. That this is highly improbable is shown by the behaviour described above of common seedlings grown from cross-pollinated seeds. The plants from which the seeds were taken were growing in two plots 40 × 9 ft. and separated from similar plots of giant sainfoin by only 6 ft.

Both varieties were in flower at the same time and there was, therefore, ample opportunity for hybridization, yet none of the next generation seedlings showed any flowering. There are three possible explanations of this:

(*a*) Giant and common sainfoin are intersterile. There is, as yet, no evidence on this point.

(*b*) The F_1 generation of a common × giant cross does not flower in its seeding year. There is, as yet, no evidence on this point.

(*c*) Hybridization did not take place.

Whichever be the true explanation, seeding year flowering in common sainfoin cannot be due to fertilization of the parent by giant pollen. The third possibility seems the most probable and is supported by the behaviour of the visiting insects. If flowers are abundant honey bees tend to work within a very small area and do not fly any distance from one plant to the next.

In the case of giant sainfoin the possibility of hybridization does not arise as seed is harvested after the second flowering and there is no chance of pollination from common sainfoin.

SUMMARY

1. The number of flowers per raceme is slightly greater in Common than in giant sainfoin.

2. Little more than half the flowers set seed under conditions of open pollination.

3. When the flowers were protected and left untouched the self-fertility was found to be 0·98 %. When the flowers were artificially self-pollinated by tripping the self-fertility was significantly higher, being 5·11 %.

4. Seedlings grown from selfed seed showed poor establishment and reduced vigour.

5. Evidence is brought forward which suggests that the phenomenon of seeding year flowering in common sainfoin cannot be due to the fertilization of the parent plants by pollen from giant sainfoin.

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ROOT ROT, SHOOT ROT AND SHANKING OF TULIP CAUSED BY *PHYTOPHTHORA CRYPTOGEA* PETHYBR. & LAFF. AND *P. ERYTHROSEPTICA* PETHYBR.

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(With Plates XXVI and XXVII)

CONTENTS

Introduction

These investigations began with the receipt of information in February 1930 from the Plant Pathological Laboratory of the Ministry of Agriculture at Harpenden that specimens of forced tulips submitted from a grower in Hampshire had been diagnosed as attacked by *Phytophthora cryptogea*. Twelve thousand out of 30,000 bulbs of the variety William Copland had failed to flower, while a large batch of variety Bartigon then being forced was unsatisfactory. The bulbs had been planted in rather heavy soil in poorly drained boxes, and the trouble had been attributed by another consultant mainly to those factors. Slugs and other pests had been troublesome, but it was possible readily to isolate a species of

Phytophthora in pure culture from some of the specimens, as well as from a few of the Wm Copland plants which had been preserved.

The first record of this disease, for which the name "Shanking" was suggested at the time, had been made by the Plant Pathological Laboratory in February 1928 (Pethybridge *et al.* 1934). Specimens of the variety Vermilion Brilliant received from Middlesex showed contraction, wrinkling and ultimate death of the bases of the flower stalks, so that the flowers failed to develop beyond the bud stage (Pl. XXVI, fig. 1). From the affected tissues *P. cryptogea* was isolated, and the identification was confirmed by Ashby (1929*b*). During the early months of 1928, and of 1929, further similar specimens were received at Harpenden from Lancashire, Scotland and other parts of the country. The variety most frequently affected was Wm Copland, but the disease was also seen in Wm Pitt, Bartigon and Tea Rose. A short account of the disease was published by Foister (1930) and, apart from a brief reference to its occurrence in the Spalding area by E. R. Wallace (1936), there is no further literature on the subject. When these researches were begun little or nothing was known about the disease. It was assumed that infection took place from the soil via the roots and that *P. cryptogea* was the cause, but no definite evidence in support of either assumption had been obtained.

During the course of this work over 10,000 experimental bulbs have been grown, and individually recorded, while observations have been made on material obtained from commercial nurseries. Shanking represents only one phase of the disease, and all stages of attack may be obtained from that of a bulb which fails to give rise to any shoot above the neck of the bulb, through that of shoots which rot off at the base before the leaves unfold, to that of typically "shanked" flowers, and even to examples where a marketable flower, probably of poor quality, is cut, although the plant contains the parasite in its roots. Further, the disease has been induced for the first time in outdoor plantings of tulips.

The experiments carried out proved not only that *P. cryptogea*, but also *P. erythroseptica*, may be the cause of the disease. Apart from obtaining negative results from a few trials with *P. cryptogea* var. *Richardiae*, and with *P. parasitica*, no attempt has been made to deal exhaustively with other species of *Phytophthora* as possible pathogens of the tulip. From time to time during the past 8 years *Pythium* spp. (Moore & Buddin, 1937) have been isolated from diseased tulips exhibiting symptoms not unlike the present disease. In the larger commercial nurseries in the Southern Advisory Province, however, the *Pythium* disease

appears to be of little importance compared with that due to *Phytophthora* spp.

It has been shown that the soil is the outstanding source of infection on the nursery. Effective means of control have been demonstrated, and the importance of putting these into practice is evident when it is realized that not infrequently houses, containing 30,000 bulbs which have been forced, produce not a single marketable flower.

Phenomena that might be confused with true Shanking. Blindness in tulips may result from too early forcing, from wide fluctuations in temperature, or too high a temperature during the forcing period, especially with "prepared" bulbs, or from forcing too small a bulb, especially if it has been badly stored, or has been damaged by *Penicillium*. Alternatively, a very mild attack of Shanking may give the grower the impression wrongly that he has been supplied with bulbs which were too small for forcing, or that they came from a "weak stock".

The flower buds may also fail occasionally through allowing the plants, or the atmosphere, to get too dry at a critical stage, while "pseudo-shanking" may result from the careless use of fertilizers.

METHODS

Isolation of the parasites. The great majority of the isolations were made by immersing cut portions of the infected material in a shallow layer of water in deep Petri dishes, which could be incubated and dealt with in large numbers. This method was particularly useful for the examination of roots, and permitted frequent observation of the material with the low power of the microscope without serious risk of contamination. Later, transfers were made from the mycelium in the water cultures to set plates of either Waksman's acid peptone agar, or of Quaker Oat agar, any excess of water having previously been drained off. At 25° C. the *Phytophthora* usually grew away readily from contaminating bacteria, etc., but sometimes transfers were made to further set plates of Quaker Oat agar before the pure cultures were tubed.

With material in an advanced stage of attack, it is usually easier to demonstrate the presence of the parasites in the roots, if any remain, than in the rotting base of the stem, owing to the presence of numerous secondary organisms in the latter.

Contamination of the soil. In pathogenicity tests, new sterilized boxes, constructed with adequate drainage, were filled with steamed soil. After a suitable interval the top layer of soil was removed and pure cultures of the strains were mixed with the soil that was to lie immediately below the bases of the bulbs. The bulbs were then planted and covered with the soil that had been temporarily removed. Liberal, approximately equal, amounts of inoculum (usually the chopped contents of 6–12 tube cultures on Quaker Oat agar to each box) were used.

Owing to the frequency of mixed infections with *Phytophthora* spp., and the likelihood of introducing *Sclerotium Tuliparum*, or *Botrytis Tulipae*, from commercial establishments, it was only rarely that contaminated soil from nurseries was used. In

testing "over-summering" of the parasites, and methods of control, soil which had been contaminated with pure cultures for one or more years was used. There was some variation in the size of boxes used and, consequently, in the number of bulbs planted in them but, throughout the experiments, the degree of crowding of the plants was comparable with ordinary commercial conditions. No difficulty was experienced in forcing successive crops of tulips in the soil.

The experimental bulbs. When necessary "own grown" bulbs were investigated but, ordinarily, imported tulip bulbs were used. The bases of the old flowering stalks, and the brown outer tunics, were removed to minimize complications from tulip fire, and to enable more careful examination for their freedom from minor troubles, such as lesions caused by *Penicillium*. No form of surface sterilization of the bulbs was attempted, but no serious complications arose from diseases that might have been carried with the bulbs. Occasionally, a shoot was infected with *Fusarium avenaceum* (Beaumont & Buddin, 1938), but fire (*Botrytis Tulipae*) occurred only to a very slight extent under glass, although it was troublesome in the tulips grown in succession out of doors. Clear-cut results were prevented in some instances by the difficulty of maintaining a uniform temperature for flowering in part of the available glasshouse. The optimum temperature for these species of *Phytophthora* is 77–81·5° F., so that a satisfactory forcing temperature of 65–70° F. would have favoured the parasites at least as much as the tulips. When the temperature could be maintained, first-class flowers were obtained as early, and as fine in quality, as those commonly marketed. It is of interest to mention the statement by Mr Geo. Shawyer (1932) that, even on his nurseries, the average waste in tulips, i.e. the actual difference between the number of blooms sold and the number of bulbs bought, was 9·12% over a period of 10 years.

The pre-forcing period. The bulbs were planted in September, and then placed in a partly shaded position, bedded in, and covered by several inches of well-washed and sieved, sterilized ashes. Spaces approximately 2 in. wide, packed with ashes, were left around individual boxes, and there was no evidence of any spread of infection from one box to another during the 2–3 months in which the bulbs were forming their root systems out of doors.

Presentation of data. A complete picture of the results of any particular experiment would require a mass of statistical data showing the amount of growth at various periods during forcing. This would be meaningless without careful standardization of the types, and weights, of the planted bulbs, and greater control of watering and temperatures in all parts of the forcing house than was possible. An "adequate" number of bulbs was always employed and, as the production of marketable blooms was considered to be the most important practical criterion, most of the results, other than descriptive ones, are presented in that form. Perhaps the greatest need for some amplification of the figures arises in connexion with Table III; in the three instances where no flowers of either variety were produced the growth of the Wm Pitt plants during the first weeks of forcing was definitely better than that of the Wm Copland. On the other hand with strain 21 of *P. cryptogea* in 1931–2 the Wm Copland plants made good foliage growth in spite of yielding only two blooms, while most of the twenty flowers of Wm Pitt were below first quality, and some of the roots were attacked. All the important experiments were replicated, and no emphasis was placed on any but obviously significant differences in numbers of flowers, after considering the growing conditions.

EXPERIMENTS WITH VARIOUS SPECIES AND STRAINS OF *PHYTOPHTHORA*

The fungus first isolated from "shanked" tulips at Harpenden in 1928 was identified as *Phytophthora cryptogea* Pethybr. & Laff. Early in 1930, amphigynous oogonia were produced in abundance on the Hampshire material, and isolations from different plants had all produced numerous oogonia on Quaker Oat agar by the following September, when one of the isolates was submitted to Mr S. F. Ashby of the Imperial Mycological Institute. The oospores were larger than those of the 1928 tulip strain of *P. cryptogea*, having a range on Quaker Oat agar from 18 to 42 μ, with an average diameter of 30·5 μ, while the oogonia ranged from 23 to 46 μ, with a mean diameter of 35 μ. The fungus was provisionally identified as either *P. cryptogea* var. *Richardiae* (Buism.) Ashby (Ashby, 1929*b*, p. 259), or *P. erythroseptica* Pethybr. (Pethybridge, 1913)[1] but the final decision was difficult, owing to different measurements having been recorded by different authors for the oospores of *P. erythroseptica*. The original material had conidia of the non-papillate type with a shallow apical, hyaline layer. Ashby measured conidia growing in a water culture at 23° C. from a young growth on bean agar—a medium giving larger and more abundant conidia than oat agar. On the fourth day twelve of the larger, fully mature, conidia—some of which showed internal cleavage into zoospores—yielded a mean of $43·5 \times 27·5\,\mu$ (length/breadth ratio = 1·58), and a range of $36–55 \times 21–33·5\,\mu$. The facile production of both sexual organs and conidia suggested *P. erythroseptica*.

In order to determine the identity of the parasite concerned inoculations were made with named species of *Phytophthora*, and further isolations were made from plants from commercial nurseries. Five strains of *P. erythroseptica* and two of *P. cryptogea* var. *Richardiae* were obtained through the courtesy of the Centraalbureau voor Schimmelcultures and of the Lister Institute. On maize-meal extract agar the Hampshire strain was indistinguishable in colony texture and hyphal character from the *erythroseptica* strains which, apart from some differences in growth rate, were similar to one another. On the other hand, the two *Richardiae* strains resembled one another but were unlike the others. This evidence

[1] Later, in February 1932, Ashby considered the strains used in these experiments in the light of *P. Drechsleri*, which had recently been described by Tucker (1931). The latter grows freely at 30° C., and will grow even at 35° C. on maize agar plates, but the tulip strain corresponded to *P. erythroseptica* (and *P. cryptogea* var. *Richardiae*) in that there was no continued growth at 30° C.

strengthened the indication that the Hampshire strain from tulips was *P. erythroseptica*.

A. The following authentic strains of *P. erythroseptica* were used in 1931–32:

(1) Isolated from Hampshire (1930) tulip material, and used by S. F. Ashby for comparative cultural work.

(2) Parallel culture to no 1, but kept continuously at Reading.

(3) Isolated from potato by G. H. Pethybridge, and originally received at the C.B.S. in January 1923.

(4) A later isolation from potato by G. H. Pethybridge, received at the C.B.S. in December 1929.

(5) Isolated from *Atropa* by Mrs N. L. Alcock (1926), and sent to S. F. Ashby by C. E. Foister.

(6) Isolated from potato by A. E. Muskett, and received by the C.B.S. in March 1930. (Found by Ashby to resemble no. 1 particularly closely.)

(7) The same strain as no. 4, but passed through potato tubers and reisolated in December 1930.

In addition, isolates numbered 8–16 that had been identified by the writer were used. They had originated from the Hampshire material; no. 14 had been in culture from the spring of 1930, while nos. 8, 9, 11, 13 and 16 had been reisolated from either Wm Copland or Wm Pitt bulbs grown indoors or out of doors in soil contaminated with these isolates during 1930–1. Nos. 10 and 15 had been isolated from bulbs adjacent to inoculated bulbs, while no. 12 was isolated from a Wm Pitt bulb grown in soil contaminated with a portion of the infested nursery soil.

The results obtained when these strains were used to contaminate the soil are given in Table I. The presence of *P. erythroseptica* was demonstrated in diseased plants from almost every box, and pure cultures were made from the majority. These cultures were used for further soil contamination, and the parasite was repeatedly reisolated. Mr Ashby examined sporangia and oospores developing from stumps of typical Wm Copland plants infected with strains 1, 4, 6 and 7: the sporangia were variable in size and non-papillate, while the oospores were always amphi-

Table I. *Numbers of marketable flowers produced in soil contaminated with the strains of* P. erythroseptica *indicated. Normally, twenty bulbs were planted in each box, and the Wm Pitt were forced several weeks later than most of the Wm Copland*

	Clean soil controls		Strain of *P. erythroseptica*															
Variety	A	B	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Wm Copland	18	17	1	5*	10	1	0**	0	2	0	0	4	2	2	1	3	19	13
Wm Pitt	20	19	1	0*	20	0	4	0	1	0	2	0	0	0	0	2	3	0

* Twenty-five bulbs per box. ** The plants in this box made very fair foliage growth, but all failed to flower owing to the disease.

gynous, and from 28 to 33 μ in diameter, with oogonia of 33–38 μ, although diminutive sizes could be found. Measurements of reproductive bodies developing on the host material from which strain 8 was isolated were: sporangia $49 \times 30\,\mu$, with a range of $35\text{–}69 \times 19\text{–}36\,\mu$ and the high length/breadth ratio of over 1·6. Oospores 25–29 μ with walls about 3 μ thick; oogonia 30–37 μ with amphigynous antheridia.

The comparatively free flowering of the Wm Copland plants in soil contaminated with strains 15 and 16 may have been due to a small quantity of inoculum, or to some unrecorded difference in the conditions. The results with strain 3 are interesting. Although all twenty of the Wm Pitt bulbs flowered successfully, sporangia of *P. erythroseptica* were obtained readily from the roots which were examined after the flowers had been cut. This strain had been *in vitro* for some 8 years; it differed from the other strains in spreading on maize agar plates more rapidly, and in forming no sexual organs on Quaker Oat agar under conditions in which the other strains produced them freely. The reaction of this strain at 30° C. was also rather different: during the first 3 days after transfer from 23° C. on maize agar the diameter of the colony increased three times as much as the other strains, although none of them continued growth at 30° C. between the third and fifth days. In dealing with *P. palmivora* Ashby (1929*a*) mentioned instances of loss of or decreased pathogenicity of strains in artificial culture for several years, while Thompson (1929) recorded that a strain of *P. Colocasiae* failed to infect after 2 years in culture although, when freshly isolated, it caused a patch canker of *Hevea* rubber.

Notwithstanding these minor irregularities, consideration of Table I, and the growth of the plants throughout the season, can leave no doubt about the pathogenicity to the tulip of authentic strains (4, 6 (Pl. XXVI, fig. 2) and 7) of *P. erythroseptica* originally obtained from potatoes, of the numerous isolates and reisolates from the Hampshire material, or even of strain 5 from *Atropa*. This was thought by Alcock (1926) to be a distinct variety, although Tucker (1931) stated that "the reasons for separating the *Atropa* strain are not very convincing, and, pending further evidence, the writer prefers to include it in *P. erythroseptica*".

B. The following strains of *P. cryptogea* var. *Richardiae* were used in 1931–2:

(17) Received from the Lister Institute.

(18) From the C.B.S. Originally received at Baarn in February 1927, having been isolated about 1926. In 1932–3 tests were made with strain 19, isolated by Miss Buisman from Calla Lily in 1930: received from the C.B.S. February 1932.

The results obtained when these strains were used for soil contamination are shown in Table II. Owing to the poor cultural conditions for the plants in these particular boxes the results in 1931–2 were not satisfactory, but no evidence was obtained that the failures were due to parasitic activity of the fungus. The results with the more recently isolated strain used in 1932–3 were satisfactory. Although more work needs to be carried out with the *Richardiae* variety of *P. cryptogea*, it appears to be either not pathogenic to forced tulips, or is of no more importance than species of *Pythium* (Moore & Buddin, 1937). Considering the above results in conjunction with those in the preceding section, it seems clear that the parasite concerned in the Hampshire nursery in the spring of 1930 was *P. erythroseptica*.[1]

Table II. *Numbers of marketable flowers produced in soil contaminated with strains of* P. cryptogea *var.* Richardiae. *20 bulbs per box*

	Season 1931–2				Season 1932–3	
	Controls in clean soil		Strain of *P. cryptogea* var. *Richardiae*		Controls in clean soil	Strain of *P. cryptogea* var. *Richardiae*
Variety	A	B	17	18		19
Wm Copland	18	17	11	16	20**	20
Wm Pitt	20	19	20	7*	19***	20

* Forced early in an inadequately heated house with large fluctuations in temperature. A comparable box of the same variety in *clean* soil gave only 11 flowers. ** Average of four boxes. *** Average of two boxes.

C. *Soil contamination with* P. parasitica *Dastur.* A strain of *P. parasitica* was isolated from tomato plants affected with foot rot in August 1935, and was used in the autumn to contaminate duplicate boxes of sterilized soil. Twenty-five Wm Copland tulips were forced in each box, and were then placed out of doors until the following autumn when the new bulblets, which appeared to be quite healthy, were removed. New Wm Copland bulbs were forced in the same soil during the following season. In 1935–6 forty-nine flowers and, in 1936–7, forty-seven flowers were cut from plants growing in contaminated soil, while twice the number of bulbs in clean soil gave ninety-nine and ninety-three respectively. From this small amount of evidence there is no reason for suspecting *P. parasitica* to be a root parasite of forced tulips.

[1] To avoid misunderstanding it may be stated here that the name *P. cryptogea* var. *Richardiae* was used in error instead of *P. erythroseptica* on p. 287 of *Reports on the work of Agricultural Research Institutes and on certain other agricultural investigations in the United Kingdom for* 1930–1, published by the Ministry of Agriculture in 1932.

D. *Soil contamination with* P. cryptogea *Pethybr. & Laff.* As the fungus first isolated from "shanked" tulips was *P. cryptogea*, and this species has also been isolated many times since from nurseries, experiments were set up to test an authentic strain of it alongside strains isolated from tulips: at the same time its effects were compared with those of *P. erythroseptica*. The following strains were used in 1931–2:

(21) Isolated from aster by L. Ogilvie, and *in vitro* for a few years.

(22) Isolated in spring 1931 from tulips on a commercial nursery in Middlesex.

In 1932–3 strains 21 R and 22 R, reisolated from the plants attacked the previous year, were used together with the following additional strains:

(23) Isolated in spring 1932 from tulips in Middlesex.

(24) Isolated from tomato plant affected with foot rot.

Strains 22 and 22 R were compared with a culture of *P. cryptogea* from Baarn, and were found to be comparable, giving non-papillate, proliferating sporangia with a length/breadth ratio of about 1·45.

The results obtained from soils contaminated with these strains are given in Table III (see also p. 708).

Table III. *Numbers of marketable flowers produced in soil contaminated with strains of* P. cryptogea. *Twenty bulbs per box, the Wm Pitt being forced after the Wm Copland*

	Season 1931–2				Season 1932–3						
	Clean soil controls		Strain of *P. cryptogea*			Strain of *P. cryptogea*					
							22 R			24	
Variety	A	B	21	22	Clean soil controls	21 R	A	B	23	A	B
Wm Copland	18	17	2	0	20*	3	0	0	0	18	13
Wm Pitt	20	19	20	0	19**	14	0	0	2	18	16

* Average of four boxes. ** Average of two boxes.

The strain (no. 24) from tomato was not so disastrous in its effects on tulips as the strains from tulip, or even the one from aster (Pl. XXVI, fig. 3). The intensification of parasitism with continued growth on the same host is no new phenomenon, and, as shown in Table III, the virulent strain 22 from tulip did not give a single flower from 120 bulbs: indeed, in the first season not a single shoot of Wm Copland appeared from twenty planted bulbs. Table III shows that *P. cryptogea* is as vigorous a parasite of forced tulips as *P. erythroseptica*. Both species have frequently been found, either independently or together, causing serious losses on nurseries every year since the work was begun, but the field of commercial growing has hardly been covered completely enough to justify

any opinion as to which species is the more commonly implicated. The greatest difference noted between the two species was the relative virulence to varieties, and this was not sufficient to be of practical importance.

Caution is necessary in diagnosing tulip diseases in the absence of pure cultures. *P. erythroseptica* may occasionally produce the proliferating sporangia shown by Pethybridge & Lafferty (1919) to be a characteristic feature of *P. cryptogea*. The presence of an odd proliferating sporangium in water cultures is not sufficient, therefore, to indicate definitely *P. cryptogea*, although the comparative frequency may be significant to an experienced observer. Roots alone rarely produce oogonia in water cultures but, occasionally, oogonia of *P. erythroseptica* may be seen from fleshy material after 24 hr. and, if the sterile water is changed to keep the cultures comparatively free from bacteria, they are usually abundant by the end of a week. This is in marked contrast to *P. cryptogea*, since it is very rare for this species to produce oogonia in water cultures, or in young pure cultures on agars.

Soil contamination as a factor in the occurrence of the disease on nurseries

The high proportion of flowers, as well as the clean roots, obtained in the controls in the experiments already described indicate that no disease was carried by those bulbs. On the other hand, the fact that both species of *Phytophthora* concerned form oospores, together with the ease with which the disease was produced when pure cultures were mixed with the soil, strongly suggests the latter as the chief means by which the disease is carried over. Nevertheless, especially in view of the disinclination of growers to accept this view, further experimental results may be quoted to emphasize the point.

In the early stages of the investigation, in May 1930, the trash and new bulblets were riddled out of a box of infected Bartigon plants brought back from Hampshire, and the soil was kept through the summer in a box exposed in the garden. In the autumn, thirty-six bulbs of Wm Pitt were planted in unsterilized garden soil which had not carried bulbs of any kind for a considerable number of years, but some of the old Hampshire soil was mixed with it just below the planted bulbs. On taking the boxes into the glasshouse there were only eight shoots, and only two of the plants ultimately produced poor flowers. At the same time numerous pure cultures of *P. erythroseptica* were obtained from the plants that had failed. On the other hand, thirty-six bulbs from the same con-

signment planted in sterilized garden soil gave a complete stand of shoots and thirty-four good flowers were obtained. That the infection came from the old Hampshire soil was clear from the fact that forty-two Bartigon bulbs in the unsterilized garden soil gave a perfect stand of shoots when taken out of the ash bed. They were forced under different conditions and only twenty-two flowers were finally cut but, in sterilized soil under the same bad forcing conditions, only eighteen Bartigon flowers were cut from an equivalent number of bulbs.

In later experiments the bulblets and trash were sometimes riddled out soon after the parent bulbs had been forced in soil contaminated with cultures, but in any event before any rooting of the bulblets had taken place, and at least a month before the new bulbs were planted. Although the boxes were given an occasional light watering during the summer, they were kept separated (to prevent infection spreading from box to box) and the soil became dry from time to time—certainly drier than it would get with the boxes stacked, or soil thrown in a heap, as under commercial conditions. The soil in each series was thoroughly mixed and filled into new, sterile boxes, before the fresh set of experiments was begun.

The first trials with soil that had previously borne an experimental diseased crop were carried out in 1931–2, but the plants met with such unusually poor growing conditions that it would be confusing to quote the actual flowering results. Only *P. erythroseptica* was concerned, and for reasons which were not clear there was a very poor "carry over" when Wm Copland was the test host. Wm Pitt grown in other portions of the same contaminated soil, however, gave numerous failures definitely due to the fungus. The results obtained in subsequent years are given in Table IV. Figures are also given for comparable bulbs planted in the same, but sterilized, lots of soil.

During 1933–4 *P. cryptogea* failed to give definite evidence of "carry over" when Wm Pitt was grown, although the crop of Wm Copland was reduced to 10%. In this instance the comparative failure of the parasite seems to be more definitely connected with the apparently lower susceptibility of the variety to the particular species. On the whole, when Table IV is considered in conjunction with Tables I and III, and supported by observations on nurseries, etc., there is abundant evidence that both species may remain virulent in the soil from one season to the next. No attempt was made to find how many years the parasites could live in soil left either in a heap or used for other crops, since the important point for the grower is the source of infection. Once it is shown that the

Table IV. *Numbers of flowers produced when tulips were grown in soils that had, during the previous season, carried plants attacked by* P. erythroseptica *or* P. cryptogea, *compared with the crops in the same soils sterilized*

Contaminant, season and treatment	Number of bulbs planted		Number of flowers cut		Percentage number of flowers cut	
	Copland	Pitt	Copland	Pitt	Copland	Pitt
A. Soil contaminated with *P. erythroseptica*						
1932–3						
Untreated	120	107	76	20	63·3	18·7
Soil sterilized	180	179	179	177	99·5	98·9
1933–4						
Untreated	40	80	2	33	5·0	41·2
Soil sterilized	40	80	38	78	95·0	97·5
B. Soil contaminated with *P. cryptogea*						
1932–3						
Untreated	20	15	1	21	5·0	60·0
Soil sterilized	20	179	18	177	90·0	98·9
1933–4						
Untreated	80	40	8	39	10·0	97·5
Soil sterilized	80	40	80	39	100·0	97·5
Untreated*	60	20	57	20	95·0	100·0

* This untreated soil was originally contaminated with the weak strain 24 from tomato. Although not very virulent to tulips, one of two tomato plants placed in the box in which a few tulips failed became attacked by foot rot.

disease can carry over in soil it is easy to point to bad practices that help the parasites to persist. The grower who allows contamination to become serious in a large bulk of bulb soil does not usually take adequate steps to prevent accidents when the boxes containing diseased plants are "run out". Moreover, it is difficult to remove all the bulblets from soil that has been used for forcing bulbs, and the longevity of a fungus in the soil depends to a large extent on whether the soil is free from "ground keepers", and even from slightly susceptible hosts. The parasites may also be carried over in the clods of old soil left in the corners of boxes. It is of interest to note that, although the disease occurs in outdoor plantings when the soil is deliberately contaminated, in bulb growing practice, where it is comparatively simple to arrange for rotation of the crop, no occurrence of the disease has been reported.

The mode of infection of bulbs planted in contaminated soil

To obtain more definite information about the way in which plants are attacked, bulbs were planted in soil heavily contaminated with *P. erythroseptica* and *P. cryptogea*, and were treated as for forcing. At intervals some of them were carefully lifted, washed and examined both macroscopically and in water cultures, etc., in the laboratory. The course

of events may be followed best from a brief description of one set of experiments using *P. erythroseptica.*

Wm Pitt bulbs were planted both in clean soil "controls" and in the contaminated soil on 17 September 1931. By 5 October, i.e. 18 days after boxing, only one out of eight bulbs examined showed any root production, while after 25 days half of the bulbs, whether in clean or contaminated soil, showed little or no root development. The optimum temperature for these *Phytophthora* spp. is 25–27·5° C., and as the soil is beginning to cool off rapidly at this time of the year it is probable that the irregularity in root production determines to some extent the degree of infection of the tulips, and accounts for the contrasts between badly diseased, and almost healthy plants that are always so perplexing to the grower: a perplexity that is increased owing to the fact that no heap of soil is ever uniformly contaminated. Some of the bulbs showed dead root tips while, in the worst affected, a number of roots were killed back to their point of attachment to the basal plate. From these roots sporangia of the parasite were obtained. A week later, 32 days after planting, there was still a great variation in the amount of rooting even in the controls. Of twelve plants from the contaminated soil, one with practically no roots, three with very short roots, and one with moderate roots showed no signs of infection; on the other hand, four of the others showed some of their roots infected and discoloured, while the remaining two not only had many roots discoloured, but the attack had also progressed into the basal plate. Indeed, in one of these bulbs the infection and discoloration had passed through the basal plate to the base of the flowering stem. Sporangia, pure cultures, and later oogonia were obtained from these six affected plants. It is not certain whether the fungus enters only through the root tips or whether it can directly infect the older portions of roots. Where, on a casual examination, the older portions only appeared to be attacked it was usually possible to trace mycelium in the tissues between the dead area and the root tip. Nevertheless, it is probable that the *Phytophthora* can gain access through wounded tissue, although the presence of many unattacked roots on a diseased bulb indicates that the uninjured, older portions of roots are more resistant. By 39 days (26 October) after planting, five of ten bulbs examined showed obvious rotting within the bulb, and the basal plate of six of them was definitely infected, and yielded abundant sporangia and oogonia in water cultures.

After 52 days (9 November) the attack on the flowering shoot had proceeded far enough to be readily seen in photographs (Pl. XXVI, fig. 4). Growth of the shoots had been arrested within the bulbs whereas, in clean soil, shoots projected $\frac{1}{2}$–1 in. above the noses of the bulbs. Abundant oogonia were again produced in isolations from the affected plants. Even 66 days after boxing the diseased plants had a reasonably good root system—a feature very frequently noted—although closer comparison showed a considerable difference in the amount of root between the infected plants and the controls. Further, many of the roots which had not rotted on the diseased bulbs were slightly discoloured and water soaked. Nevertheless, three out of ten plants from the contaminated soil were still apparently healthy inside. When two boxes of the same variety in equally contaminated soil were taken into the glasshouse each showed five reasonably good shoots from twenty bulbs, but all ten of these shoots failed subsequently, only one of them developing as far as producing a dried up flower bud; whereas, in clean soil, thirty-eight bulbs gave thirty-six flowers.

The experiment was repeated during the following season with both Wm Copland and Wm Pitt, and with *P. cryptogea* also as a soil contaminant. The results were similar, and no essential difference could be observed between the mode of infection of the two species. Infection has never been found to occur through the shoot above the nose of the bulb, and the experiments confirmed the method of infection deduced from examination of diseased plants from nurseries.

Symptoms after transfer to the glasshouse

A brief description may be given of the symptoms after transfer to the glasshouse. With a very virulent strain, or when the soil is heavily contaminated, there may be no healthy shoots in a box when the outdoor covering is removed, but frequently a comparatively full stand develops, and there is nothing abnormal to suggest that the money spent on fuel and housing is likely to be wasted. During the 4–6 weeks of the forcing period some of the shoots remain almost stationary, but gradually become more unhealthy in appearance until they can be twisted readily out of the bulbs, and show shrivelling and rotting of the tissues at the base. Few or many of the plants will often appear to be unfolding their leaves normally, and it is not until the first flower buds are showing colour that the tips of many of the leaves may fade, frequently with the development of red pigment, and the grower realizes that the plants are in a typically "shanked" condition. When the plants are lifted many of the roots remaining are yellowish. It is common on nurseries for some of the root stumps to be strongly discoloured, but this more intense browning seems to be due to the action of secondary organisms. In the same way there may be some rotting of the bulb scales, but this is not typical of *Phytophthora* attack *per se*, although the primary parasite often destroys completely the developing new bulb. This point, and the contrast between the rapid and complete rotting of some tissues at certain times, and the apparent barriers to progress in some of the older tissues, will be mentioned later in dealing with the symptoms in outdoor plantings. It should be stated here, however, that under experimental conditions a striking feature is the solidity and freedom from rotting of the scales of the parent bulb, even after many weeks in the glasshouse, and despite the complete rotting of the flowering shoot within the bulb. Indeed, by chance a box of bulbs that is so badly diseased that no flowers are produced may yield a larger crop of new bulblets than one which has all the flowers and foliage cut for market.

SPREAD OF THE DISEASE FROM ARTIFICIALLY INFECTED BULBS

Although a physiological disease, "topple", has symptoms resembling the tulip blossom blight attributed to *P. Cactorum* (Stevens & Plunkett, 1925), the latter has not been observed in this country, and there has been no indication that *P. erythroseptica* and *P. cryptogea* can infect the green stem of the tulip, or that aerial spread of infection can occur.

In the autumn of 1930, Wm Pitt, Wm Copland and Bartigon bulbs were planted in clean soil, and six widely separated bulbs in each box were inoculated with *P. erythroseptica* through deep wounds towards the centre of the basal plate. In some bulbs the wound was so situated, and the rot spread so rapidly, that no roots were formed, while in most bulbs the attack was serious enough to prevent the appearance of any shoot. The results may be illustrated best by giving some details of the Wm Pitt bulbs, the variety showing the most serious effects. Thirty sound bulbs were planted so that twenty-six of them were adjacent to one or other of the six inoculated bulbs. All had produced good shoots by the time they were taken into the glasshouse, while only one side shoot marked the positions of the six inoculated tulips. It was clear from isolations that the failures were due to *P. erythroseptica*, and that the disease which developed subsequently in some of the adjacent plants was also due to this fungus. The amount of spread during the forcing season was, however, comparatively limited. None of the plants showed the very poor growth so characteristic of bad outbreaks of the disease; only three "shanked", and three affected at the base of the stem gave poor flowers. Two other plants failed to give a satisfactory bloom, but there were similar failures due to factors other than the parasite in bulbs of the same variety in clean soil. In spite of the presence of six active centres of the disease, thirty bulbs gave twenty-two flowers of reasonable quality as compared with thirty-four from the larger number of thirty-six bulbs in clean soil.

During the following season two or four bulbs in the middle of boxes of Wm Copland and Wm Pitt in clean soil were inoculated with either *P. erythroseptica* or *P. cryptogea*. The inoculated bulbs usually failed completely, and though the pathogen was reisolated from an occasional neighbour the number affected was usually small. The worst instance of spread was again with the variety Wm Pitt in forty-four bulbs planted around four bulbs which failed completely after being inoculated with *P. erythroseptica*; forty of the forty-four uninoculated bulbs gave flowers, though four of them were of poor quality.

From 1931 to 1934 other experiments were carried out in which "mummified" bulbs, that had been rotted by the *Phytophthora* spp. in outdoor plantings, were placed at the centre or corners of boxes of bulbs in clean soil. On several occasions sufficient infection of the roots of neighbouring plants was obtained to show that the fungus was active in the "mummified" bulbs but, even in the worst box, only one neighbour "shanked".

Evidence is also available from other sources, since it has been noticed that, in experiments, one plant may gradually fail from the day it is taken indoors, though all its neighbours may produce good blooms.

Experimental production of the disease in outdoor tulips

All the evidence of the disease in outdoor plantings has been obtained from the experimental plots. The disease may have been overlooked out of doors because its symptoms are less severe, and also because they can so readily be confused with those of plants suffering from a basal plate damaged before planting, from careless hoeing in the early stages of growth, or from the attacks of mice or slugs. The lower temperatures prevailing during the growing season for garden tulips probably minimize the effects of the disease, while rotation of soil can be practised more readily than by many large forcers.

Sound bulbs were planted at the end of October in soil which had not carried bulbs for many years, and on some of the plots the soil was contaminated by putting several pieces of agar from pure cultures of either *P. erythroseptica* or *P. cryptogea* at the bottom of the dibble hole. In 1930–1 every bulb produced a shoot above the surface of the ground despite the fact that the depth of planting was several inches more than for bulbs in boxes. Before flowering, however, about one in eight of the Wm Pitt plants contaminated with *P. erythroseptica* showed dwarfing of the foliage to varying degrees, accompanied by conspicuous reddening (Pl. XXVII, fig. 1). In 1931–2, either through using larger quantities of inoculum, or because the winter was milder, there were numerous failures to appear above ground by early March in the Wm Pitt bulbs where *P. erythroseptica* was the contaminant, and as many as thirty-seven out of eighty-two bulbs failed thus. In no season, however, was there any appreciable number of "gaps" with Wm Copland bulbs contaminated with either fungus, or with Wm Pitt bulbs in soil contaminated with *P. cryptogea*. A cold spring followed and little change was seen until the foliage began to redden towards the end of April, when some of the plants were markedly dwarfed. The most serious case was that of the Wm Pitt

plants infected with *P. erythroseptica* already mentioned: in one row of forty-one bulbs there were twenty-one complete failures, three failed before flowering time, seven gave poor quality blooms, and only ten flowered satisfactorily.

Allowing for the lower temperature conditions, there is no essential difference between the progress of the disease in the open and indoors. Infection begins in the roots and passes through the basal plate to the flowering stem (Pl. XXVII, fig. 2), producing all stages of failure from a complete "miss" to a plant which produces a good flower, but later gives diseased new bulbs. The parasites were repeatedly reisolated, and the isolates used to reproduce the disease. The new bulbs sometimes failed early in the growing season, as already mentioned, but commonly large new bulbs were produced some, but not all, of which rotted completely by the time of digging, or occasionally after a period in storage, but before the new bulbs were finally cleaned. These infected bulbs, which gradually dried out to a "mummified" condition, were produced in both varieties irrespective of the parasite involved, and they were used in experiments described above.

In certain tissues the progress of the rot is sometimes comparatively rapid, but there are barriers to the extension of the rotting that are not completely understood. It is a common experience that the new bulb is sound although the flowering stem failed, or that the chief new bulb, and some of the offsets are completely rotten, while other adventitious bulbs are quite healthy, and remain healthy even if left in the old trash in store for a time. McKenny Hughes (1934, p. 118) refers to "the time at which the new bulbs and offsets become cut off by dead tissue from the still growing and flowering stem which perishes with the season of flowering", and it is probably this "dead tissue" which forms a barrier to the mycelium of the *Phytophthora*, in much the same way as it was thought to be a barrier to virus infection.

VARIETAL SUSCEPTIBILITY

Many more bulbs of Wm Copland are forced than any of the other varieties. Further, it is on the nurseries forcing large numbers of the popular varieties that the disease is more likely to be perpetuated. These factors, coupled with the variation in the intensity of contamination from place to place, and time to time, make it difficult to assess exactly the available evidence on the relative susceptibility of varieties. Amongst the Darwin varieties, Wm Pitt and Wm Copland, which have been used chiefly in these experiments, are highly susceptible. Rose Copland and

Bartigon are also attacked freely and Pride of Haarlem and Madam Krelage to a less extent, but Clara Butt appears to be relatively resistant. Among the Early Single varieties the disease has been seen in Vermilion Brilliant, White Swan and White Hawk, and the Double Early variety Tea Rose is also susceptible. In contaminated plots out of doors the May-flowering Cottage Tulip variety Inglescombe Yellow seems to be very resistant.

If there is any difference between the pathogenicity of the two species of *Phytophthora*, it is probably more apparent in the varietal reaction than in other features of the disease. The tentative opinion of the writer is that Wm Pitt is less susceptible to *P. cryptogea* than to *P. erythroseptica*, while, although the evidence is not so consistent, it is possible that the reverse holds with Wm Copland.

Possibility of transmission with the bulb

When a bad outbreak occurs it is, perhaps, natural for the grower to attribute it to the purchase of infected bulbs, which are usually imported. Nevertheless, no satisfactory evidence has been obtained to indicate that the bulb is ever responsible for a bad attack. Repeated appeals have been made to growers to send suspicious specimens culled from imported bulbs. Apart from occasional specimens attacked by *Penicillium*, and others damaged by exposure to hot sun at lifting time, or by some other circumstance, the only diseased bulbs that have been received proved to be infected with *Pythium* (Moore & Buddin, 1937). Growers familiar only with the Shanking phase conclude that the bulbs must be faulty when they find occasional "misses". These investigations have proved, on the contrary, that such complete failures may occur when the healthiest bulbs are planted, if the attack originates from contaminated soil. Growers are also prone to conclude that it is only the batch of plants from one particular source that is affected, whereas careful inspection usually reveals mild attacks in plants from other consignments.

In experimental plots a large proportion of the affected new bulbs are completely rotten before the crop is lifted, while those which may rot subsequently are picked out during the cleaning and sorting operations. The action of the two species of *Phytophthora* is very similar to that of one of them, viz. *P. erythroseptica*, when it produces Pink Rot of the potato (Cairns & Muskett, 1933; Pethybridge, 1913). Cairns & Muskett (1933) stated that tubers, scarcely to be regarded as so free from soil and the possibility of attached oospores as tulip bulbs, will, if affected, "have become mummified by planting time and obviously unfit for use as

'seed'". Each tulip bulb is handled as much, and by equally intelligent labour, as a potato "set", and it is unlikely that affected bulbs would be used. Further, if bulbs bearing oospores of the fungi should be planted, it is clear from the experiments on the spread of the disease from an inoculated bulb that the effect is hardly likely to approach in seriousness the bad attacks frequently seen. One grower stated in 1930 that "in every case in the centre of the affected area of tulips" (forced in boxes) "there was one dead rotten bulb, and infection from this appears to have radiated to the extent of 8–10 in., killing, or partly destroying, the bulbs within that area. In short, one bulb which was dead when planted (*sic!*) and never showed any signs of life had killed about 50 others round it." The improbability of this statement is evident from the experiments recorded in this paper.

Experiments, carried out with entirely negative results on the point at issue, may be described briefly. In 1934 a grower submitted a sample of suspected bulbs. The thirty bulbs in clean soil produced twenty-nine good flowers; the remaining bloom was imperfect, but the plant was healthy. On the other hand, 5–10% of the plants were badly affected with *P. cryptogea* when the main batch of bulbs was planted in the grower's own soil. Previous experiments included growing the new bulblets derived from experimental and commercial diseased plants. No signs of the disease were seen, although, under identical conditions, similar bulblets grown in soil contaminated with pure cultures showed abundance of the disease. Bulbs of Wm Copland, Wm Pitt and Bartigon have been raised repeatedly in contaminated soil, the infectiveness of which has been demonstrated by some of the crop being diseased. Such bulbs have been forced or grown out of doors without obtaining any evidence that the parasites might be carried on bulbs subjected to the customary cleaning and grading operations.

Positive evidence may yet be obtained that the two species are occasionally carried on the cleaned, and much handled, bulbs, but there seems little doubt that soil contamination is responsible for severe outbreaks.

CONTROL OF THE DISEASE

The possibility of contaminated water supply

With diseases caused by certain species of *Phytophthora* the possibility of a contaminated water supply must always be considered. On a nursery where one of the earliest severe attacks occurred the water was examined at the time of the outbreak by the method of Bewley &

Buddin (1921) with entirely negative results. On one nursery where there had been repeated attacks the water supply may have been contaminated, since it was drawn from an open stream, and from a point *below* the spot on the waterside where the soil and refuse from previous diseased tulips had been dumped, but the water was only one of the probable sources of infection even on that particular nursery.

Common pitfalls in connexion with soil

It has already been pointed out that soil brought in from a distance is not necessarily free from parasites such as *P. erythroseptica* and *P. cryptogea*. Contamination of "new" soil with the latter is known to cause outbreaks of "damping off" etc. in tomato and other plants, while the use of soil that has previously grown a crop of tomatoes is an obvious way in which this disease may be initiated in tulips.

The advice commonly given that tulips "should follow potatoes, or some crop that has been well manured" (Hall, 1929, p. 196) also needs to be accepted with caution. Pink rot of potatoes is not a common disease in this country, but it does occur from time to time, and may easily be overlooked in the field, although the warmer conditions of the forcing house would probably bring it into greater prominence if the fungus were introduced in the soil. Cairns & Muskett (1933) found that satisfactory potato crops can be grown in soil contaminated with the pink rot organism, "provided that strongly sprouted tubers are planted, and that soil drainage is sufficiently good to prevent the land becoming unduly wet in times of heavy rainfall". How far similar reasoning may apply to the tulip disease is not known, but there seems little doubt that the heavy soil, careless watering and poor drainage of the boxes provided by some growers may aggravate the disease when the soil is contaminated. Further, the danger of contaminating imported, clean soil must be emphasized.

Partial sterilization of soil

(i) *Use of formaldehyde.* As soon as it was realized that infection could come from the soil, and a supply of suitable soil was available, the effect of soil treatment with formaldehyde was tested. Both species of *Phytophthora* were investigated, and the experiments were continued for more than one season. The strength usually recommended for soil sterilization (1 part of freshly purchased (38–40%) formaldehyde solution to 49 parts of water) was used though, on account of the difficulties of treating small quantities in boxes, in amounts more liberal than the

standard dose. The soil was treated at least a fortnight before planting, and the boxes were covered with sacking steeped in the solution for two days after the actual treatment. Both ordinary commercial formaldehyde, and Steriform were employed, but no difference was noted in the effects, and the results are considered together (see Table V and Pl. XXVII, fig. 3). They were generally satisfactory, the treated soil giving an average of 97 % of good flowers, while only 30 % of marketable blooms was obtained from the same batches of soil left untreated. In 1936–7, when the treatment was, perhaps, carried out less carefully, only twenty-two flowers were obtained from twenty-five Wm Copland bulbs, and definite infection was present in the failures. This emphasizes the need for the greatest care in carrying out soil treatment, and in preventing re-contamination, but does not detract seriously from the value of formaldehyde in combating the *Phytophthora* disease. On the other hand, formaldehyde is not a panacea for tulip diseases in the forcing house, as was shown by work on Grey Bulb Rot (Buddin, 1937). Further, as Bewley (1935) states—"failure of chemical sterilizers is a constant feature of heavy soils, and there is little doubt that it is due to the impossibility of making intimate contact between the sterilizing agent and every particle of soil".

(ii) *Other chemicals.* It was suggested by Foister (1930) that the tulips should be watered with a solution of Cheshunt Compound as soon as the disease was observed. The investigation of the mode of infection made it clear, however, that such late soil treatment would be useless, though it did not rule out Cheshunt Compound for use at, or before, the time of planting.

In a preliminary experiment in 1931–2, soil contaminated with *P. erythroseptica* was watered with Cheshunt Compound several weeks before planting, or immediately after covering the bulbs with soil. The conditions for flowering were unsatisfactory, but there was clear evidence from the pure cultures isolated that some of the failures were due to *Phytophthora*. Further, when the soil was not treated until immediately before the boxes were covered with ashes, there was some evidence that the chemical itself affected the plants adversely under the rather static conditions for soil aeration and moisture during the rooting period. Such action is, perhaps, less likely under modern commercial conditions where the boxes are covered with straw.

The results obtained in subsequent years are summarized in Table V. The standard strength of Cheshunt Compound was used, but sometimes as much as twice the quantity ordinarily recommended was applied.

Table V. *Flowering in contaminated soil compared with that in the same soil when steam sterilized, or treated with formaldehyde (or Steriform) or Cheshunt Compound*

			Untreated control		Steam sterilized soil		Soil treated with formaldehyde or Steriform		Soil treated with Cheshunt Compound	
Season	Soil contaminant	Variety	No. of bulbs planted	No. of flowers cut	No. of bulbs planted	No. of flowers cut	No. of bulbs planted	No. of flowers cut	No. of bulbs planted	No. of flowers cut
1932–3										
	P. erythroseptica	Pitt	107	20	59	59	120	118	40	11
	P. erythroseptica	Copland	120	76	60	59	120	120	40	30
	P. cryptogea	Copland	20	1	—	—	20	18	—	—
1933–4										
	P. cryptogea	Copland	80	8	20	20	60	60	60	18
	P. erythroseptica	Copland	40	2	20	20	20	18	20	8
	P. erythroseptica	Pitt	80	33	20	20	60	58	60	41
1936–7										
	P. erythroseptica and *P. cryptogea*	Copland	50	12*	25	25	25	22	—	—
	Totals		497†	152†	204	203	425	414	220	108
Percentage flowering			30·6†		99·5		97·4		49·1	

* A dozen flowers of very poor quality were produced in addition in this batch.

† The totals and the percentage flowering in the actual controls for each treatment are slightly different, but the variation in the percentage flowering is only from 30·6 to 32·5.

Table V shows that, although the Cheshunt Compound had a slight fungicidal effect, yet less than one half of the bulbs bloomed satisfactorily. The failures being due to the parasites, Cheshunt Compound cannot, therefore, be recommended even if the treatment be carried out before planting.

Other chemicals were not tried extensively. One proprietary article was tested on duplicate boxes of contaminated soil, but the control obtained was not as good as with formaldehyde, and the cost would be greater.

(iii) *Use of steam.* The value of steam sterilization is clearly shown in the results summarized in Table V. The contaminated soil averaged little more than 30% of flowers, while the same soil, subjected to routine steam sterilization, gave 99·5% of marketable blooms (Pl. XXVII, fig. 4). Although steam is of almost universal value for the eradication of disease when the value of the crop justifies its cost, growers are sometimes sceptical of the type of growth which may result. No statistical data can be presented, but it may be said that in no instance did steaming the soil—carried out a fortnight or so before planting—have any adverse effect on growth: on the contrary, the largest plants and the finest blooms were invariably produced.

SUMMARY

Phytophthora erythroseptica and *P. cryptogea* have been isolated on numerous occasions from diseased tulip plants on commercial nurseries, and the isolates, both in pure culture and in their effect on the host, have been compared with authentic cultures of these fungi. Both species have been proved to be, either separately or in conjunction, the cause of a serious root rot, shoot rot and shanking of forced tulips.

Infection, which takes place from contaminated soil through the roots, begins as soon as the first roots are produced from the basal plate of the bulb. The parasite passes directly through the basal plate to the base of the flowering stem, leaving the old bulb scales practically unattacked. According to the time, or rapidity, of infection an affected bulb may produce no visible shoot, or it may give a shoot bearing a flower bud that remains blind because the flower stem is rotted at the base (shanking). Sometimes a bulb with some of its roots attacked may produce a marketable flower of poor quality.

A very similar disease has been induced in outdoor tulips by contaminating the soil.

The disease, which may result in the total loss of a forced crop, has not yet been reported outside Great Britain, where it seems to be widely distributed.

There is no evidence that the disease is carried with the planted bulb in ordinary commercial practice, and the rate of spread in the glasshouse from one or more inoculated bulbs planted in the midst of healthy ones is comparatively slow.

The susceptibility of varieties of tulips to the two species of *Phytophthora* is briefly discussed.

A contaminated water supply is a possibility, but appears to be relatively unimportant.

Partial sterilization of the contaminated soil by steam, or formaldehyde, combined with hygiene, is an effective method of controlling the disease, but soil treatment with Cheshunt Compound is unsatisfactory.

Certain strains of *Phytophthora cryptogea* var. *Richardiae* and *P. parasitica* were tested, but appear to be of little, or no, consequence as parasites of forced tulips.

A large proportion of the success of the present investigations has been due to the stimulus, and ever-ready helpfulness, of Dr Geo. H. Pethybridge and Mr W. C. Moore. The credit for identifying the fungi concerned lies almost entirely with Mr S. F. Ashby. The writer also wishes to express his thanks to his Laboratory Attendant, Mr A. Sherval, for taking the photographs.

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Fig. 1

Fig. 3.

Fig. 4.

Fig. 2.

BUDDIN.—ROOT ROT, SHOOT ROT AND SHANKING OF TULIP CAUSED BY *PHYTOPHTHORA CRYPTOGEA* PETHYBR. & LAFF. AND *P. ERYTHROSEPTICA* PETHYBR. (pp. 705–729)

Fig 1

Fig 3

Fig. 2.

Fig 4

BUDDIN.—Root Rot, Shoot Rot and Shanking of Tulip caused by *Phytophthora cryptogea* Pethybr. & Laff. and *P. erythroseptica* Pethybr. (pp. 705–729).

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EXPLANATION OF PLATES XXVI AND XXVII

PLATE XXVI

Fig. 1. A "shanked" Wm Copland tulip with shrivelled flower bud and poorly developed, fading foliage consequent on attack on the roots by *Phytophthora cryptogea*; the bulb has been cut to show that the rotting has proceeded through the basal plate and affected the base of the flowering stem which is slightly discoloured. The original specimens for which the name "shanking" was suggested had probably been attacked earlier, or by a more virulent strain, and showed more shrivelling of the base of the flowering stem than the plant illustrated.

Fig. 2. Wm Pitt tulips in soil contaminated with *P. erythroseptica* (*a*, strain 1 from tulip; *c*, strain 6 from potato) contrasted with plants from the same number of bulbs in similar, but clean soil (*b*). Photographed 22 February 1932.

Fig. 3. Wm Copland tulips (*a*) in soil contaminated with *P. cryptogea* (strain 21 from aster) compared with plants (*b*) grown under the same conditions in clean soil. Photographed 26 January 1932.

Fig. 4. Bulb of variety Wm Pitt (*a*) cut 52 days after planting in soil contaminated with *P. erythroseptica*; the roots and basal plate are attacked, as also the base of the flowering stem. The bud which should eventually form the new bulb is completely rotten. Bulb from clean soil (*b*) for comparison. Photographed 9 November 1931.

PLATE XXVII

Fig. 1. Wm Pitt tulips in outdoor plot in soil contaminated with *P. erythroseptica*. Note the stunting of the affected plants, much of the foliage of which had reddened. Photographed 9 May 1931.

Fig. 2. Wm Pitt tulip bulb planted out of doors on 30 October 1931 in soil contaminated with *P. erythroseptica*. The rot has progressed from the roots up the flowering shoot, but the old bulb scales are not attacked. Photographed 21 March 1932.

Fig. 3. Wm Copland tulips in soil contaminated with *P. cryptogea* that had produced a diseased crop during the preceding season. *a*, soil treated with formaldehyde several weeks before the planting date; *b*, soil left untreated. Photographed 31 January 1933.

Fig. 4. Wm Copland tulips in soil from a nursery contaminated with both *P. erythroseptica* and *P. cryptogea*. (*a*) soil steam sterilized; (*b*) soil left untreated. Photographed 9 January 1937.

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INVESTIGATIONS ON THE RUST OF ROSES *PHRAGMIDIUM MUCRONATUM* FR.

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CONTENTS

INTRODUCTION

THE rust of roses caused by *Phragmidium mucronatum* has of recent years become increasingly prevalent, especially on stocks grown for grafting. Where a severe attack occurs on such plants, retardation of growth due to premature defoliation and to the death of branches infected by the caeoma stage may result in heavy financial loss to the grower. It has also been suggested that roses on an infected nursery may contract the disease from the infected briars and be responsible for its introduction into gardens hitherto free from it.

At the request of the National Rose Society, an investigation of this disease was commenced in 1933. The work has been concerned mainly with two problems, the possibility of cross-infection from the briar to the rose, and overwintering. Certain other questions have also received attention.

Phragmidium mucronatum produces all spore forms. The aecidiospore stage is found mainly on buds and stems where it often forms large caeomata. It may also occur on leaves. It apparently plays a large part in the overwintering of the fungus. On the leaves the uredospore stage is most common and is responsible for the spread of the rust during the summer. The teleutospores arise in the uredosori in the autumn and

uredospores and teleutospores are commonly found together in the same sorus. In the late autumn teleutospores predominate and may be the only spore form present.

Both aecidiospores and uredospores have been found to infect suitable hosts readily. Eriksson (1924) reports that aecidiospores from overwintered mycelium on *Rosa rubrifolia* failed to infect, while aecidiospores from fresh infections did so. This observation has not been confirmed in the present investigation. In one case a plant which showed caeomata in 1935 produced a further zone on the same branch in 1936 below that of the previous year, although it had not been exposed to fresh infection. Spores from this second caeoma caused infection. The mycelium had, in this case, been at least two years in the plant.

Conditions for the natural spread of the fungus would appear to be unfavourable at Cheshunt. Infected plants of *Rosa canina* were planted on the nursery in 1933. A certain amount of spreading to neighbouring plants of *R. laxa* occurred in 1934 but the disease gradually died out, although, in order to avoid possible loss of caeomata, the plants were not pruned. Attempts to infect roses and *R. laxa* in a large experimental glasshouse were also unsuccessful. In one experiment, plants placed on the bench immediately after inoculation became infected, but usually it was found necessary to place the plants in a moist chamber to secure infection.

Experiments were carried out to determine the minimum time of exposure to moist conditions which would result in infection. Using aecidiospores from Kokulensky's *canina* as inoculum, plants of *R. laxa* kept in the moist chamber overnight became infected. A similar result was obtained with uredospores of the same strain. The actual period in the moist chamber was 13–14 hr. Infection would probably follow much shorter exposures to moist conditions since, in one experiment, a plant became infected after only 4 hr. in the moist chamber. This experiment was, however, carried out by day when it proved impossible to maintain a saturated atmosphere in the moist chamber for more than a short period. The results were therefore inconclusive.

The failure of the fungus to infect save in the moist chamber had the incidental advantage that cross-infections between strains from different sources were very rare, only two cases occurring among over 500 plants inoculated.

Strains of the fungus

(*a*) *Infection experiments*

Many rose growers believe that rust on the cultivated rose has become more widespread since the use of *R. laxa* as a stock has become more common. It has been suggested that the stock may influence the reaction of the rose to the rust, but no evidence in favour of this hypothesis has been obtained in the present investigations. Alternatively, it has been maintained that a new race of *P. mucronatum* has been introduced on *R. laxa*. This race is said to be different from the native form on *R. canina* and capable of infecting the rose.

The first evidence that there might be physiological forms of *P. mucronatum* was obtained in 1934, when repeated attempts to infect *R. laxa* with rust from the cultivated rose failed. Roses inoculated at the same time were freely attacked. It was decided to carry out comparative experiments with rust from different sources, using as test plants the cultivated rose, *R. laxa* and several strains of *R. canina*. By the courtesy of Mr Courtney Page pot plants of the following were obtained.

(*a*) Deegen's *canina*.
(*b*) Kokulensky's *canina*.
(*c*) Schmidt's Special.
(*d*) *R. canina* (unnamed strain).
(*e*) *R. laxa*.
(*f*) Rose Margaret McGredy on seedling briar.
(*g*) Rose Margaret McGredy on *R. laxa*.

During 1935, a series of inoculation experiments was carried out using the following strains of the rust:

A. Uredospores on leaves of *R. canina*.
B. Uredospores on leaves of *R. laxa*.
C. Uredospores on leaves of cultivated rose.
D. Uredospores on leaves of *R. rugosa*.
E. Aecidiospores on stems of *R. canina*.
F. Uredospores on leaves of *R. laxa* (a second collection).
G. Aecidiospores on leaves of cultivated rose.
H. Aecidiospores on buds and stems of Kokulensky's *canina*.
K. Aecidiospores on buds and stems of Deegen's *canina*.

Strain D from leaves of *R. rugosa*, unfortunately died out before the full series of test plants was available, and only its reactions on the rose and *R. laxa* were tested. Several of the strains (those from

briars) ceased production of uredospores at the end of the year and it was impossible to keep them alive over the winter. Those on the rose, however, survived and it was possible to reisolate strains E, H and K from caeomata on their respective hosts in the following spring.

In each experiment all the test plants were inoculated at the same time. Spores were transferred to the healthy leaf by means of a fine needle. The topmost four or five fully developed leaves on a growing shoot were inoculated, thus eliminating the effect of the age of the leaf on infection. The inoculated shoot was marked and the remainder of the plant served as control. In order to ensure adequate exposure to moist conditions the inoculated plants were kept in the moist chamber for 72 hr. and inoculations were made in the evening so that the falling temperature would cause almost immediate saturation of the atmosphere and avoid desiccation of the spores. The plants were subsequently placed on the bench in the glasshouse, each strain being isolated by curtains of cheesecloth. In most cases each experiment was repeated several times.

The reactions of each strain are given in detail below. The reactions of the two roses were identical and they are not referred to separately although they were always tested.

A. *Uredospores on leaves of* R. canina.

This strain never produced very abundant infection even on its original host out of doors. In experimental transfers it caused slight infection with very small sori on *R. laxa*, Deegen's *canina* and the unnamed *R. canina*. The sori were often paler in colour than those of other strains, probably owing to paucity of spore production. It did not infect the cultivated rose, Kokulensky's *canina* and Schmidt's Special. The feebleness of its growth both in the glasshouse and on *R. canina* out of doors prevented more than one complete test.

B. *Uredospores on leaves of* R. laxa.

Yellow marks were produced on the upper surface of the leaves of the rose in one experiment out of four. It would appear that penetration took place to some extent, but there was no definite infection. Positive infection with spore production occurred on all the other hosts. The sori on all except Schmidt's Special were about 0·5 mm. in diameter and orange yellow in colour. On Schmidt's Special the sori were very small but numerous and bright yellow in colour. On *R. laxa*, Deegen's *canina* and Kokulensky's *canina* yellow areas appeared on the upper surface of the leaf corresponding in position to the sori on the lower.

C. *Uredospores on leaves of the cultivated rose.*

The cultivated rose was readily infected by this strain. On Schmidt's Special necrotic areas with a few very small sori were produced in one experiment; in a second, necrotic areas only appeared; while, in a third, no effect was observed. The other briars were not infected in three trials, although roses inoculated at the same time were attacked. In one experiment, a plant of Kokulensky's *canina* showed rust on both

noculated and control leaves. This rust could not be transferred back to the rose and was, doubtless, a chance infection by one of the briar strains.

D. *Uredospores on leaves of* R. rugosa.

As mentioned above, this strain, which was collected in the summer of 1933, died out before it could be tested on the full range of hosts. During 1933 and 1934, however, six attempts were made to infect *R. laxa* with it. All failed, although inoculations of the rose made at the same time were successful.

E. *Aecidiospores on stems of* R. canina.

The fungus derived from the caeomata on the *R. canina* plants at Cheshunt was more vigorous than that from the leaves of the same plants (strain A), although, doubtless, it was the same. Reddish brown marks were sometimes produced on rose leaves but no spores. Kokulensky's *canina* was not infected in five trials. Schmidt's Special was infected once out of four trials, Deegen's *canina* in five out of six, and the unnamed *R. canina* in three out of four. Only on Deegen's *canina* were the sori of normal size.

F. *Uredospores on leaves of* R. laxa.

In August 1935, leaves of *R. laxa* were received from Mr Courtney Page which were heavily infected by rust. The reactions of this strain differed markedly from those of strain B from the same host. On the rose, one very small sorus appeared on one plant in the first test, the second plant showing light green to yellow marks on the inoculated leaves. In a second trial no apparent effect was produced. On *R. laxa*, infection was very heavy when the strain was new and spore production was very abundant; there were yellow spots on the upper surface of the leaf. On Deegen's *canina* there were numerous small sori in two experiments and, on the unnamed *R. canina*, a few small sori in the first trial. Kokulensky's *canina* and Schmidt's Special were not infected. Unfortunately, the lateness of the season prevented more than two tests and the rust did not survive the following winter.

G. *Aecidiospores on leaves of cultivated rose.*

The reactions of this strain were identical with those of strain C, also from the rose. Small sori were again produced on Schmidt's Special, from which it was transferred successfully back to the rose.

Table I. *Host range of strains of* P. mucronatum

Strain	Source	Host: Rose	*R. laxa*	Kokulensky's *canina*	Deegen's *canina*	Schmidt's Special	*R. canina*
A	*R. canina*	−	+	−	+	−	+
B	*R. laxa*	?	+	+	+	+	+
C	Rose	+	−	−	−	+	−
D	*R. rugosa*	+	−				
E	*R. canina*	?	+	−	+	+	+
F	*R. laxa*	+	+	−	+	−	+
G	Rose	+	−	−	−	+	−
H	Kokulensky's *canina*	?	+	+	+	+	+
K	Deegen's *canina*	?	+	+	+	+	+

? = Necrotic lesions without spores.
+ = Infection with spore production.
− = No apparent effect.

H. *Aecidiospores from buds on Kokulensky's canina.*

This strain was identical in its reactions with strain B from *R. laxa*. It produced necrotic lesions on the rose but no spores.

K. *Aecidiospores from buds on Deegen's canina.*

The host range and reactions of this fungus were also identical with those of strain B.

The host range of these strains is summarized in Table I.

On the basis of these experiments the strains may be arranged in five groups:

(1) Strains A and E.
(2) Strains B, H and K.
(3) Strains F.
(4) Strains C and G.
(5) Strains D.

The fungi of group 1 resemble rather closely those of group 2, differing only in their inability to infect Kokulensky's *canina*. Strain D from *R. rugosa* is placed provisionally in a separate group for lack of information as to its host range. Probably it should be grouped with strains C and G from the rose. It was unfortunate that it proved impossible to obtain plants of *R. rugosa*, since tests on that host would have been of great interest.

The rose appears to be highly resistant to infection by the forms occurring on briars. In only one case (strain F) were spores produced and then only one very small sorus was formed. Allen (1927) reports a very similar situation with *Puccinia triticina* form 11 on the resistant Malakoff wheat. In that case the first invaded cells usually die immediately and the rust fails to gain a lodgement. Occasionally, however, the rust survives and forms haustoria. Feeble uredosori may even be produced. In the susceptible Little Club wheat on the other hand the host cells are not damaged until the infection is quite old and sporulation is normal (Allen, 1926). The infection of Schmidt's Special by the rose strains would seem to be on the same plane. It appears that this variety is somewhat less resistant to these strains than the other briars tested. The small size of the uredosori of the rusts of group 2 on this host suggests that it is also somewhat resistant to them. So far as these experiments go, therefore, it does not appear that cross-infection between briars of the *R. canina* and *R. laxa* groups and the rose takes place to any extent.

(*b*) *Morphological characters of the strains*

The physiological differences between the strains are, to some extent, correlated with morphological differences. Macroscopic examination did not reveal any significant variation among them when growing on suitable hosts. Measurement of the uredospores did not produce any useful information. It was found that the spores swelled in water and discharged their contents through the germ pores with a resultant secondary shrinkage. Measurements were therefore unreliable, since it was impossible to determine at what stage in this process a spore might be.

Measurements of the teleutospores, however, have given some interesting results. One hundred spores from each strain (except D) were measured. Only the actual body of the spore was considered, the pedicel and hyaline tip being neglected. The results are shown in Table II. Since the number of cells in the spore directly affects its length, the average length of each "cell class" is given separately. While no counts of fields were made, these figures give a fairly accurate idea of the proportion of each "cell class". The range of each class is shown in Table III. The width of the spores varies from 15 to 29 μ and is approximately the same in all strains.

Table II. *Average length of teleutospores in strains of* P. mucronatum

Measurements in microns

No. of cells	A	B	C	E	F	G	H	K
3	—	—	—	—	—	57·8 (1)	—	—
4	—	57·8 (2)	60·0 (1)	—	—	61·7 (8)	—	—
5	62·9 (3)	69·1 (8)	69·7 (19)	64·4 (1)	—	70·9 (30)	64·4 (3)	63·2 (7)
6	73·4 (19)	75·3 (22)	83·9 (48)	73·1 (12)	74·4 (2)	84·0 (49)	73·6 (23)	71·0 (19)
7	82·9 (51)	83·4 (46)	96·3 (32)	81·5 (53)	83·8 (25)	95·4 (12)	82·6 (51)	81·8 (48)
8	95·6 (26)	92·5 (20)	—	89·7 (34)	92·7 (41)	—	95·4 (21)	92·1 (23)
9	111·1 (1)	104·4 (3)	—	—	100·8 (30)	—	103·3 (2)	101·5 (3)
10	—	—	—	—	111·1 (2)	—	—	—

Numbers in brackets represent numbers of spores measured.

In strains A, B, E, H and K seven celled spores were predominant, in strains C and G from the rose six-celled, while strain F showed a large number of seven, eight and nine-celled spores, with eight cells the most numerous.

Table III. *Range in length of teleutospores in strains of* P. mucronatum

Measurements in microns

No. of cells	A	B	C	E	F	G	H	K
4	—	53·3–62·2	—	—	—	53·3–68·9	—	—
5	62·2–64·4	62·2–73·3	64·4–77·8	—	—	66·7–75·5	62·2–66·7	60·0–71·1
6	66·7–82·2	68·9–86·7	77·8–95·5	71·1–75·5	71·1–77·8	73·3–93·3	66·7–77·8	66·7–77·8
7	73·3–91·1	71·1–93·3	88·9–106·7	71·1–88·9	77·8–91·1	88·9–104·4	75·5–91·1	75·5–91·1
8	86·7–100·7	86·7–100·0	—	82·2 97·8	84·4–100·0	—	88·9–104·4	82·2–100·0
9	—	102·2–106·7	—	—	93·3–106·7	—	97·8–108·9	100·0–104·4
10	—	—	—	—	108·9–113·3	—	—	—

When the average length of spore in each class is considered, it is evident that with one or two exceptions the briar strains resemble each other closely while the spores of those from the rose are somewhat larger. In the case of spores with seven cells these differences were examined statistically. Twenty-five measurements were selected at random from each strain, except G, where the measurements were too few, and tested by the method outlined by Fisher & Wishart (1930). This examination showed that the average length of seven-celled spore in strain C was significantly different from those of corresponding spores in the briar strains, while the briar strains did not differ significantly among themselves. Grove (1913) figures teleutospores on Dog Rose and Burnet Rose which show similar variations in size. He regards them as belonging to the same species. Arthur (1929), however, considers the rusts on cultivated rose and certain wild roses to be distinct species. It is evident from the experiments described above that extensive collections of rust must be made and tested on a wide range of hosts before the exact status of these forms can be decided.

Overwintering

Phragmidium mucronatum is able to pass the winter in two ways, by means of teleutospores and as mycelium in the tissues of the host. Both of these stages appear to be of importance in the life history of the fungus.

(a) Germination of teleutospores

A period of dormancy is necessary for the proper ripening of the teleutospores of many rusts although the length of this period varies greatly in different species. Experiments have been carried out to determine its duration in *P. mucronatum* and how far it is affected by conditions of storage.

In conducting germination tests the following method was adopted. Shallow vulcanite rings were cemented on to glass slides rather towards one end. Spores were floated on distilled water contained in these rings and, also, placed dry on the slide to be moistened by the condensation water. The slides were placed in Petri dishes lined with moist blotting paper.

In the first experiment in 1933–4 leaves of *R. canina* bearing teleutospores were gathered at the end of August. It was decided to store them in glass jars with loose fitting covers and, since it had been found that fresh leaves so enclosed rapidly decayed, they were first air dried in the laboratory. One jar was kept in the laboratory while the other was placed out of doors in a glass-sided box which was open on one side. The top of this jar was protected from possible wetting by rain by a piece of oiled silk. The average weekly temperature and humidity from the commencement of germination tests were calculated from continuous records. In the laboratory the weekly average temperature varied from 55·2° F. in December 1933 to 70·8° F. in the last week of the experiment in July 1934. Over the whole period from October 1933 to July 1934 the temperature averaged 59·6° F. Outside, the lowest temperature occurred in November and December 1933, the lowest weekly average being 35·3° F. The highest, in July 1934, was 71·0° F. and the average over the whole period 49·0° F. Humidity records were not taken over the whole period in the laboratory, but averaged 48% during the period from October 1933 to March 1934. The corresponding figure outside was 77 and 74% over the whole period.

Spores were tested at fortnightly intervals from 30 October to 26 June 1934. Slides from each batch were placed under a range of conditions of lighting and temperature, in incubators at 25, 20 and 15° C., on the bench at room temperature, and in a glass-sided box on the bench which was controlled thermostatically at about 20° C. The slides were kept under observation for a long period, the majority until the end of the experiment on 18 July. In no case was any germination observed.

For the next season, a different method of storage was adopted. A supply of rose leaves bearing teleutospores was received from Mr

Courtney Page at the end of August 1934. They were divided into three lots and each lot was enclosed in a muslin bag. One bag was hung up in the laboratory, one in a glasshouse, and the third in the glass box out of doors. A further supply, received a little later, was placed in a desiccator over water and kept on the bench in the laboratory. The average conditions of temperature and humidity from October 1934 to September 1935 in these positions is shown below.

	Temperature ° F.			Humidity %		
	Weekly average		Whole period	Weekly average		Whole period
	Max.	Min.		Max.	Min.	
Laboratory	72·4	55·9	63·0	80·2	42·8	58·0
Glasshouse	76·0	56·7	66·1	87·6	71·2	79·0
Outside	72·5	40·6	53·8	83·2	60·2	71·9

Germination tests were commenced on 8 October 1934 and continued until 12 September 1935. The attempt in the previous year to investigate the effect of temperature and light as well as of conditions of storage on germination had led to an unwieldly accumulation of slides and, therefore, it was decided to make the tests at room temperature only. Tests were made at monthly intervals until germination was first observed at the beginning of May. They were then made at approximately weekly intervals for a short period.

Germination was observed in the four tests made on 8, 14, 20 and 29 May. In each case the spores which germinated had been stored outsid and were floating on water. No spores stored in the laboratory or in the glasshouse germinated, nor did any that had been placed dry on the slide. Of the very large number of spores used in each test only a very small proportion germinated, those tested on 14 May showing the largest number. The promycelia varied greatly in length and were colourless, except where a little of the yellow spore contents had been held up by the septa. The sporidia were bright yellow.

In 1936, teleutospores on rose exposed out-of-doors again showed germination during May.

From these experiments, it appears that the teleutospores of *P. mucronatum* require a period of rest and that exposure to winter conditions is necessary for their proper ripening. Exposure to cold alone does not appear sufficient, since the winter of 1933–4 was appreciably colder than the following one. In the winter of 1934–5 the spores, while protected from direct wetting by rain, were exposed to mist and fog, and it may be suggested that the preliminary drying and storage in jars in the previous year led to the death of the spores. It must, however, be

acknowledged in comparing the two years that the teleutospores from *R. canina* were of a different strain from those used in the following year and that the strain was not very vigorous, as shown above.

The small proportion of spores which germinated may, perhaps, be due to the conditions of the experiment. Possibly the leaves were picked before many of the spores were fully developed and only a small proportion was able to mature during the winter. A number of the riper spores may also have fallen off during the process of sampling. Possibly, teleutospores developing and passing the winter under natural conditions would show a higher percentage of germination, and would also germinate over a longer period.

(*b*) *Infection experiments with teleutospores*

Numerous attempts have been made to infect rose bushes with rust by means of the teleutospores. In addition to the more usual method of hanging leaves on or over the plants, bushes have been atomized with spore suspensions and infected leaves have been placed on the soil. Inoculated plants have been kept in the moist chamber for varying periods or placed immediately on the bench. In no case has any infection taken place though, in experiments made during May 1935, spores from the leaves used for inoculation showed a small percentage of germination.

The writer has been informed by Dr Deacon of Norwich that he has seen teleutospores adhering to the stems of rose bushes by their mucilaginous pedicels. In the germination experiments described above only spores floating on water germinated and it may be hazarded that, in nature, a film of rain water may persist in cracks in the bark or between bud scales long enough to enable spores there lodged to germinate and to infect the plant. Dr Deacon's observations suggest that the use of a winter wash may be of value in preventing this source of infection. Caeomata may also occur on leaves but, so far, the method by which this type of infection is brought about has not been elucidated.

(*c*) *Perennial mycelium*

If, as seems likely, the teleutospores only germinate during early summer, the mycelium must be capable of surviving the winter in the stem since caeomata have been observed as early as 28 March. These caeomata continue to produce spores for some weeks but, in most cases, the branch is killed and the fungus dies. A number of pot plants of Kokulensky's *canina* developed caeomata in 1935, and were segregated and kept under observation. In all cases except one the infected branches were killed and, in the following year, the plants remained healthy. In

one plant, a second zone of caeomata developed in 1936 below the portion of stem infected in the previous year. This plant again produced a diseased bud in the autumn of 1937 on the same branch and below the previous lesions. At no time did the leaves of the plant show any infection, so that the mycelium must have travelled to a small extent in the tissues of the host. There seems, however, to be no evidence of systemic infection such as has been found in the Blackberry in America (Dodge, 1923). There seems little reason, therefore, to fear the passage of rust from an infected stock to the rose grafted on it. Stems showing caeomata should be cut out as soon as observed since it appears that this spore stage is, probably, the most important in starting an epidemic of the disease.

Summary

1. Cross-inoculation experiments have shown that *Rosa laxa* and certain varieties of *R. canina* are not attacked by *Phragmidium mucronatum* from the cultivated rose, and that the rose is resistant to certain forms of the rusts on briars.

2. These differences are correlated with differences in the size of the teleutospores.

3. The teleutospores of *P. mucronatum* require exposure to winter conditions before they will germinate.

4. The mycelium of the rust may persist for several years in the stem but there is no evidence of systemic infection.

The writer is indebted to Mr Courtney Page for supplying material and for much valuable information, and to Dr W. F. Bewley for helpful criticism and advice.

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SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT

III. DECOMPOSITION OF THE RESTING MYCELIUM OF *OPHIOBOLUS GRAMINIS* IN INFECTED WHEAT STUBBLE BURIED IN THE SOIL

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(With Pl. XXVIII and 3 Text-figures)

CONTENTS

I. Introduction

In the first two papers of this series (Garrett, 1936, 1937), the relation of soil conditions to the subterranean spread of *Ophiobolus graminis* along the roots of wheat plants was examined; evidence was presented in support of the hypothesis that advance of the fungus was mainly controlled by the accumulation of respiratory carbon dioxide in the immediate neighbourhood of the root. On this hypothesis, the antagonism of the other soil micro-organisms to the parasitic activity of *Ophiobolus* would be represented chiefly by their contribution to soil respiration which, in turn, would raise the concentration of carbon dioxide in the neighbourhood of the root.

Soil conditions, however, affect also the second phase in the life history of *Ophiobolus*, viz. the decline in viability of its resting mycelium in the infected root and stubble residues left in the soil after harvesting the crop. The conditions of temperature, moisture and aeration which at one time

[1] The greater part of this work was carried out during the tenure of a Leverhulme Research Fellowship.

favour the rapid subterranean advance of the fungus along the roots of the growing crop may, at another time, bring about its rapid disappearance from infected plant residues buried in the fallow soil. Biological control of *Ophiobolus* by the other soil micro-organisms is exercised more, perhaps, through the direct decomposition of its resting mycelium than through the retarding effect of soil respiration upon its activity in the parasitic phase.

It has been suggested (Garrett, 1938*a*) that this alternation between an active parasitic phase on and in the underground parts of the host plant, and a resting or declining phase in the soil may be a comparatively general characteristic of the more highly specialized root-infecting fungi. Such fungi appear to be confined to existence upon their host plants, not through inability to live as saprophytes, since the majority are readily cultured both upon the ordinary laboratory media and upon sterilized soil, but on account of the rigorous competition for existence upon the decomposing organic matter of the soil. The useful distinction between obligate parasites and facultative parasites is, thus, of limited value when applied to the soil-borne fungi; to the small class of obligate parasites, such as *Plasmodiophora*, which cannot be cultured for apparently nutritional reasons, must be added a larger class behaving as obligate parasites in the soil, for reasons of biological competition.

The effect of soil conditions upon the incidence and distribution of the take-all disease of wheat is thus a problem which will not be solved before both phases in the life history of the fungus have been studied adequately; a preliminary investigation of the resting phase has now been completed, and is presented in this paper.

The decomposition of dried and powdered fungus mycelium by the soil microflora has been studied by Heck (1929), Jensen (1932), and Norman (1933). The present work appears to be the first reported on the decomposition of the resting mycelium of a parasitic fungus in the soil. The "soil plate" method, devised by Cholodny (1930), and used by Conn (1932), Jensen (1934–6) and others, has demonstrated very convincingly the rapid decomposition of vegetative fungus mycelium in the soil. The addition of such a supplement as rye-grass meal or dried blood to the soil results in a rapid multiplication of the soil fungi, the best development of which on the Cholodny slides is seen after a period of some 7–10 days at a temperature of 15–20° C.; after some 10–14 days, the fungus mycelium begins to disappear comparatively rapidly from the slides. The relation between bacteria and the decomposing hyphae is especially well seen; the sites of past hyphae on the slides are frequently

marked out by the almost continuous development of bacteria. A detailed study of the parasitism of the common soil-inhabiting fungus *Trichoderma lignorum*, upon another soil fungus, *Rhizoctonia solani*, has been made by Weindling and his collaborators (1932–6). No apology need therefore be made for the working hypothesis to be adopted in this paper, viz. that the disappearance of the resting mycelium of *Ophiobolus* from infected wheat stubble is due primarily to its direct decomposition by the soil microflora.

II. Methods

The experiments to be described have been made with artificially infected wheat stubble; the artificially infected material has the advantages over the naturally infected stubble of greater uniformity, and of being obtainable in desired amount and in a fresh condition for experiments at any time of the year. It is intended, however, to confirm all major conclusions by corroborative experiments with the natural material. The general plan of the experiments has been as follows. The pieces of artificially infected wheat straw, each some 2·5 cm. in length and thoroughly impregnated with *Ophiobolus* mycelium, have been buried in the different experimental soils in lots of 50; glass tumblers of the type previously used (Garrett, 1936) have again been employed as soil containers. Every fortnight, a sample of two tumblers, containing 100 straws in all, has been taken from each batch of soil, and the straws tested for viability of the contained *Ophiobolus* mycelium. A simple cultural test for viability, i.e. by surface sterilization of the straw and plating out on an agar medium, has not, unfortunately, been feasible. *Ophiobolus*, though not a slow-growing fungus, is easily suppressed on an agar medium by other fungi present in the infected plant tissue. The wheat seedling itself has, therefore, been adopted as the ideal selective medium for *Ophiobolus*. The infected straws have been washed free of soil, split slightly, and a wheat seed inserted in each. The straw plus seed has then been planted in sand, which affords ideal infection conditions for *Ophiobolus* (Garrett, 1936). The percentage of straws containing viable *Ophiobolus* mycelium has then been given by the number of infected wheat seedlings eventually obtained. Methods and materials employed in these experiments may now be described in more detail.

Preparation of the infected straw was made as follows. From a complete wheat straw, the nodes were cut out with scissors, each with 2–2·5 cm. of internode straw attached. One cut was made immediately below the node, the other 2–2·5 cm. above it; the internode straw consisted of the true stem together with the enclosing leaf-

sheath. Inclusion of the comparatively resistant node served to hold the straw together during the subsequent period of decomposition in the soil. A preliminary sorting of the straws was made before cutting, and those of external diameter less than 3–4 mm. were rejected. The batch of cut straws for an experiment was first boiled for 3 hr. in a decoction of string beans at the rate of 1 lb. of string beans in 4 l. of tap water to every 4000 straws. After being left overnight in the decoction, the wet straws were allowed to drain for a brief period, and were then sterilized in lots of 1000 or so in 2 l. Erlenmeyer flasks, for a period of $1\frac{1}{2}$ hr. at $1\frac{1}{2}$ atm. in the autoclave. Each flask of straws received as inoculum some 300 g. of a freshly grown culture of *Ophiobolus* on sand +3% corn-meal medium, the contents of a single 500 c.c. Erlenmeyer culture flask. The finely dispersed corn-meal-sand inoculum was well shaken up with the straws, which thus received a simultaneous and uniform inoculation. The flasks of inoculated straws were generally incubated for a period of about 1 month at 25° C. After this period of incubation, the cells of the straws were thoroughly impregnated with *Ophiobolus* mycelium. The nitrogen content of the infected straws after incubation averaged over several experiments some 0·4% of dry matter. At the conclusion of the incubation period, the straws were washed free from the adhering remains of the sand and corn-meal inoculum over a sieve, and counted in lots of fifty into small envelopes.

Soils for the experiments were air-dried shortly before use, and passed through a 1·8 mm.-mesh sieve (14 meshes to the inch). Each batch of soil was thoroughly mixed before use, and the moisture capacity at saturation determined by the perforated box method of Keen & Raczkowski (1921). Tobacco tins 2·25 cm. in depth and of some 200 c.c. capacity were found convenient for this purpose. Since the saturation capacity varies considerably with the physical state of aggregation of the soil, it was found essential to make a fresh determination for each batch of air-dried soil. The glass tumblers used as soil containers were 8·5 cm. in height by 6·75 cm. internal diameter, and were filled with dry soil to a capacity of some 180 c.c. (representing a depth of 5 cm. of dry soil). An addition of 1 g. of fertilizer per tumbler represents a dressing of 1 ton 2·5 cwt. per acre mixed with the top 2 in. of soil, or one of 3 tons 7·5 cwt. per acre mixed with the top 6 in. of soil.

In preparation for an experiment, the weight of 180 c.c. of air-dry soil was determined for each soil, and this amount weighed out per tumbler for each series. The volume of water required to bring the moisture content of each soil to 50% or other desired degree of saturation was next calculated. All tumbers had been previously numbered with their own weights, and the stock arranged in weight classes, so that the heavier tumblers could be employed for the lighter soils and vice versa. It was thus generally possible to bring some 500 tumblers, constituting a single experiment, to not more than two final weights, which greatly facilitated the periodical waterings. The supplements to be added to each soil series, if insoluble or incompletely soluble, were mixed beforehand with the air-dry soil, but if soluble were added as a solution at the time of setting up the experiment. The experiments were generally designed to run for a period of 18 weeks, so that 18 tumblers were required for fortnightly samplings in duplicate from each soil.

In setting up an experiment, the tumblers were filled as follows. The 50 straws were tipped into the bottom of the tumbler and some three-quarters of the air-dry soil added; the top of the tumbler was closed with the palm of the hand, and straws and dry soil mixed up by brisk rotatory shaking. The remaining quarter of dry soil was

added finally to cover any straws lying on top. After the 18 tumblers of one series had thus been filled with soil, the correct volume of distilled water (or solution) to bring the soil to 50% (or percentage desired) saturation was added from a measuring cylinder. If the water was added to the centre of the soil in each tumbler, i.e. at one spot only, and not too quickly, no trouble was experienced with air-locks, and a rapid and uniform percolation resulted. The straws helped to keep the soil more open and thus, no doubt, assisted percolation of the water; it was not found necessary to continue with the more cumbersome method of filling and watering the tumblers as employed in the previous work (Garrett, 1936). By 48 hr. after filling, the distribution of moisture was comparatively even, as judged by soil colour; indeed, the difficulties of moisture distribution were largely avoided by working with these small quantities of soil. Only where moisture contents in the neighbourhood of 30% saturation were employed was it found desirable to mix soil and water by hand in a basin before filling into the tumblers. The tumblers were brought to the appointed final weight by the addition of some 100 g. of a mulch of moist sand (at 50% saturation).

An experiment comprised some 20–30 series of eighteen tumblers each, and the setting up was spread out over 2 days, which made the subsequent work of sampling more easily managed. The tumblers were kept on trestle tables in the open laboratory, the temperature of which (recorded by thermograph) varied from 15–22° C. during winter and summer, with an average temperature of approximately 18° C. Tumblers were arranged not in series but randomized on the tables, to cancel out any possible place effect. The sand mulch greatly reduced water loss by evaporation; this was made good on a box balance once a week. Tumblers of pure sand lost water much more rapidly, and these were generally watered in mid-week from a constant-delivery pipette on a rubber hose.

Sampling was done at fortnightly intervals from 2 to 18 weeks. The ball of soil from each tumbler was knocked out on to a sieve, and the infected straws sieved free of soil in water. Each straw was split halfway down from the upper (or internode) end with two cross-cuts of a scalpel, and a Little Joss wheat seed inserted. The fifty straws from each tumbler, each enclosing a wheat seed, were planted in stencil-made holes in a wooden seedling flat of pure sand, in five rows of ten each, 3 cm. between seedlings and 4 cm. between rows. The sampling and planting was done by three persons, the first washing out and splitting the straws, the second inserting the wheat seeds, and the third doing the actual planting in the flats of sand. The flats were stacked in the laboratory (mean temperature 18° C.) for 1 week, until seedlings were emerging, and were then transferred to the glasshouse (20–30° C.) for a further 2 weeks' growth of the young plants. Seedlings were washed out from the flats of sand 3 weeks after planting. No nutrient solution was found necessary for this limited period of growth, and the sand was steamed and used repeatedly.

Determination of infection was made by inspection of each wheat seedling floated out in water over a white-enamelled tray. The binocular dissecting microscope was used at first to confirm the presence of the runner hyphae of *Ophiobolus*, but it was soon found that proximal discoloration of the seedling roots was a reliable criterion of the presence of the fungus, under the standardized conditions of these experiments. This method is not claimed to be an infallible test for the presence or absence of viable *Ophiobolus* mycelium in the infected straws. But sand is a rooting medium more favourable for infection than any naturally occurring field soil (Garrett, 1936),

and the roots of the seedling are enabled to explore the infected straws very effectively (see Pl. XXVIII, which shows examples of severely infected and healthy seedlings respectively; all gradations between the two are naturally found). Moreover, at the time of washing out of the seedlings, infection had generally advanced some 4–5 cm. or more along the roots, so that nothing would have been gained by a longer growing period. Since the spread of the fungus from one seedling to another can occur only through root contact (Garrett, 1936), the 3 weeks' growing period represented a satisfactory compromise, giving ample time for the development of infection, but not enough for appreciable spread of the fungus from one seedling to another by root contact.

It will be understood that a seedling having visible discoloration on the proximal part of one only, or more, of its roots was regarded as infected by *Ophiobolus*; the straw through which it had grown was therefore recorded as still containing viable mycelium of the fungus. Figures for percentage viability of *Ophiobolus* given from the following experiments are thus based on the wheat seedling test of 100 pieces of infected straw, derived from a sample of 2 tumblers taken fortnightly from each soil series. It must be emphasized that percentage viability of the fungus as indicated at any time by this test is necessarily greater than the total percentage of mycelium still viable in the mass of 100 infected straws regarded as a whole; the terms "50% viability" and "100% viability", where subsequently used for the sake of brevity, must therefore be taken as referring strictly to the percentage of straws containing viable fungus.

III. EXPERIMENTAL

The experiments will be numbered serially and described in chronological order:

Exp. I. The following series of experimental soils were set up:

Series 1–4. Slough soil air-dry and at moisture contents of 30, 50 and 80% of saturation, respectively. The soil was obtained from the Biological Field Station of the Imperial College of Science and Technology at Slough,[1] and is similar to that used in the previous work with *Ophiobolus*. The air-dry stock prepared for this experiment had a saturation capacity of 60 c.c. per 100 g. of air-dry soil; the *p*H value was determined by quinhydrone electrode as 7·2. The soil at 30% saturation was mixed with water by hand before weighing out into the tumblers.

Series 5–8. Inoculated sand air-dry and at moisture contents of 30, 50 and 80% saturation respectively. The saturation capacity of the sand was determined as 22 c.c. per 100 g. of air-dry sand. The series at 30, 50 and 80% saturation were inoculated with the microflora of Slough soil, prepared by shaking up 200 g. of moist screened soil with 1 l. of water; the suspension was allowed to settle for 1 min., and then decanted and used for the watering of the sand. Unfortunately, the heaviness of the inoculation thus increased with the moisture content of the sand, but the possible importance of actual numbers of organisms added was not appreciated when the experiment was set up. The series at 30% saturation was mixed with water by hand.

Series 9 *and* 10. Inoculated sand and Slough soil, respectively, +3% of rye-grass meal (nitrogen content 1·2% of dry matter).

[1] By courtesy of Prof. W. Brown.

Series 11 *and* 12. Slough soil treated with 0·3% of sulphuric acid and with 0·25% of calcium hydroxide, respectively, by weight of the air-dry soil. The soils were mixed with the respective chemicals at a moisture content of 40% saturation, incubated in loosely-covered glass containers for 15 days at laboratory temperature (18° C.), and then air-dried before use. The *p*H values of the acid-treated and limed soils were electrometrically determined as 5·8 and 8·3, respectively.

Series 13 *and* 14. Series 13 was Slough soil at 50% saturation maintained under improved conditions of aeration in 4-in. unglazed flower pots, of approximately the same capacity as the glass tumblers. Additional water had to be added (22 c.c. per pot) to compensate for that taken up by the porous walls. Series 14 was Slough soil at 50% saturation, in tumblers kept in a loosely-covered glass jar inside an incubator maintained at 23° C., i.e. under very still and uniform conditions.

Series 15. Infected straws kept air-dry in the absence of soil in empty glass tumblers.

The results of this experiment are given in Table I and are shown graphically in Text-fig. 1.

Table I. *Percentage viability of* Ophiobolus *resting mycelium in infected straws*

	Weeks								
Soil series	2	4	6	8	10	12	14	16	18
No. 1, soil, dry	100	100	100	100	100	100	100	100	100
,, 2, soil, 30% sat.	100	98	99	77	72	26	13	9	1
,, 3, soil, 50% sat.	100	86	90	74	61	47	22	36	4
,, 4, soil, 80% sat.	100	97	92	76	64	24	44	19	58
,, 5, inoc. sand, dry	100	100	100	100	100	100	100	100	100
,, 6, inoc. sand, 30% sat.	100	100	98	85	100	84	100	76	39
,, 7, inoc. sand, 50% sat.	100	100	100	99	96	90	56	50	3
,, 8, inoc. sand, 80% sat.	100	98	87	94	97	27	59	51	18
,, 9, inoc. sand, +3% rye-grass	100	97	84	6	4	0	—	—	—
,, 10, soil + 3% rye-grass	100	49	21	2	0	0	—	—	—
,, 11, soil + 0·3% sulphuric acid	100	86	82	17	2	0	—	—	—
,, 12, soil + 0·25% calcium hydroxide	100	86	63	21	1	0	—	—	—
,, 13, soil at 50% sat. in 4 in. [illegible]	100	96	81	58	50	45	5	19	0
,, 14, soil at 50% sat. in closed incubator	100	95	92	74	68	77	71	71	47
,, 15, straws kept air-dry in empty tumblers	100	100	100	100	100	100	100	100	100

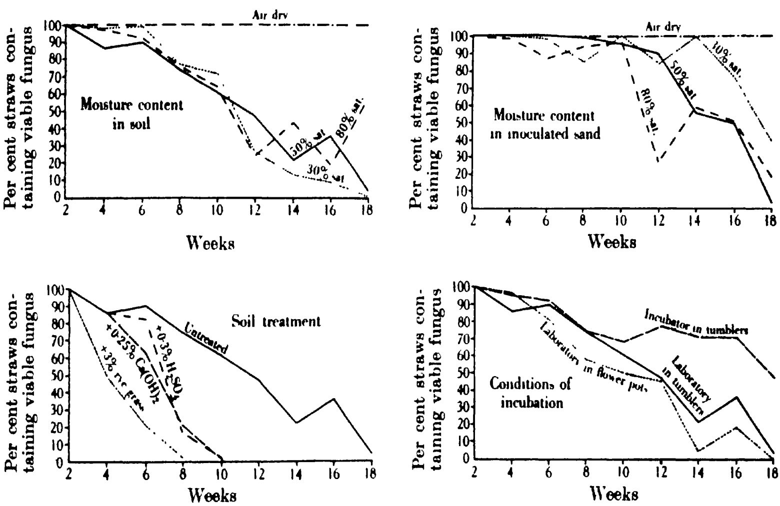
The following conclusions may be drawn:

(i) The viability of the fungus persisted apparently undiminished (i.e. as shown by this test) throughout the experimental period of 18 weeks in dry air, sand and soil.

(ii) In the moist soils, variation in moisture content over the range 30–80% of saturation did not appreciably affect the decline of the fungus, either in Slough soil or in inoculated sand. The decline was less regular at 80% saturation than at the two lower moisture contents.

(iii) In the three Slough soil series, 50 % loss of viability occurred at 10–12 weeks, as against 16–18 weeks in the three inoculated sand series. It appears that the better aeration in the inoculated sand series did not compensate for the much lower numbers of micro-organisms initiating decomposition in this series.

(iv) The addition of 3 % of rye-grass meal greatly accelerated loss of viability both in Slough soil and in the inoculated sand. This may be attributed tentatively to increase in the numbers of soil micro-organisms through multiplication on the added organic material and, thus, to a more rapid attack on the *Ophiobolus* mycelium in the infected straw.



Text-fig. 1. Decline in viability of the fungus in the soils of Exp. I.

(v) Pre-treatment of the soil both with lime and with sulphuric acid accelerated the disappearance of *Ophiobolus*. Discussion of this result will be postponed for the production of further data in Exp. II.

(vi) Loss of viability in the flower-pot series appears to have proceeded at a slightly but uniformly greater rate than in the corresponding tumbler series. In the series maintained in a lightly covered glass vessel in the incubator, loss of viability at first kept pace with that in the control series kept in the open laboratory, but then slowed down abruptly after 8 weeks. Whereas 50 % loss of viability occurred in the open laboratory control at 12 weeks, it was deferred until 18 weeks in the incubator series. It may be suggested that improved aeration in the flower pot series has

increased microbiological activity, and hence loss of viability of *Ophiobolus*, whereas decreased aeration in the incubator series has correspondingly reduced it. The maintenance of uniform conditions in stored soil is known to lead to reduction of microbiological activity to a comparatively low level (Waksman & Starkey, 1923), and it is probable that other factors besides a retarded gas exchange in the incubator series have contributed to the observed result.

Exp. II. All soils in this experiment were started and maintained at a moisture content of 50% saturation; the following series were set up:

Series 1–8. Different soil types.

Series 1. Carbello, *p*H 4·9, an organic loam.

Series 2. Tunstall, *p*H 5·5, a sandy heath soil.

Series 3. Rothamsted, *p*H 6·8, a clay loam.

Series 4. Woburn, *p*H 7·2, a light loam, previously well limed.

Series 5. Slough, *p*H 7·2, a medium loam.

Series 6. Downham, *p*H 7·5, a black fen soil.

Series 7. Whitchurch, *p*H 8·1, a clay loam on chalk subsoil.

Series 8. Aldeburgh, *p*H 8·1, a sandy heath soil, well limed.

Series 9. Slough soil, kept in refrigerator at 2–3° C.

Series 10 *and* 11. Slough soil, pre-treated with 0·3% sulphuric acid and with 0·25% calcium hydroxide, respectively, exactly as in Exp. I, to give soils of *p*H values 5·8 and 8·2, respectively.

Series 12. Slough soil, steamed moist for 3 hr., air-dried and reinoculated by mixing with a tenth part by weight of the unsterilized soil.

Series 13–17. Slough soil with 3% by weight of the following organic supplements, starch, rye-grass meal, corn-meal, rape dust and dried blood.

Series 18–21. Slough soil adjusted to different C/N ratios by addition of 5 g. glucose per tumbler, corresponding to 2·5 g. glucose or to 1 g. carbon per 100 g. dry soil, together with variable amounts of ammonium carbonate to give C/N ratios of 100, 50, 10 and 5, respectively.

Table II. *p*H *values of soils from Exp. II*

	Weeks								
Soil series	2	4	6	8	10	12	14	16	18
No. 5, untreated Slough	7·2	7·1	7·1	—	—	—	—	—	—
„ 13, +3% starch	7·4	7·5	7·3	7·5	7·6	7·5	7·7	7·6	7·4
„ 14, +3% rye-grass	7·3	7·2	7·3	7·3	7·3	7·3	7·2	—	—
„ 15, +3% corn-meal	7·3	7·4	7·2	7·2	7·2	7·1	7·1	6·9	6·9
„ 16, +3% rape dust	7·0	5·0	4·8	5·0	4·9	4·8	4·9	4·9	4·9
„ 17, +3% dried blood	7·7	5·7	5·0	5·0	4·7	4·7	4·5	4·4	4·5
„ 18, C/N 100	7·3	7·0	7·2	7·3	7·3	7·3	7·0	7·1	7·1
„ 19, C/N 50	7·2	6·3	6·6	6·7	6·8	6·7	6·7	6·6	6·5
„ 20, C/N 10	8·3	5·0	5·1	5·2	5·4	5·3	5·5	5·4	5·3
„ 21, C/N 5	8·6	7·2	5·9	5·2	4·9	4·9	4·9	5·0	5·0

The *p*H values of soils to which the various organic supplements had been added, and of those with adjusted C/N ratios, were determined periodically by means of the quinhydrone electrode, and are given in Table II.

It will be observed that a considerable fall in pH value eventually follows the addition of rape dust and of dried blood, both with a high nitrogen content, and that this occurs also in series 20 and 21 with C/N ratios of 10 and 5, respectively. This increase in soil acidity is to be attributed to the rapid progress of nitrification in the absence of a sufficient reserve of base for neutralization of the nitric acid produced.

The results of this experiment are given in Table III and Text-fig. 2. The following conclusions may be drawn from the data there presented:

Table III. *Percentage viability of* Ophiobolus *resting mycelium in infected straws*

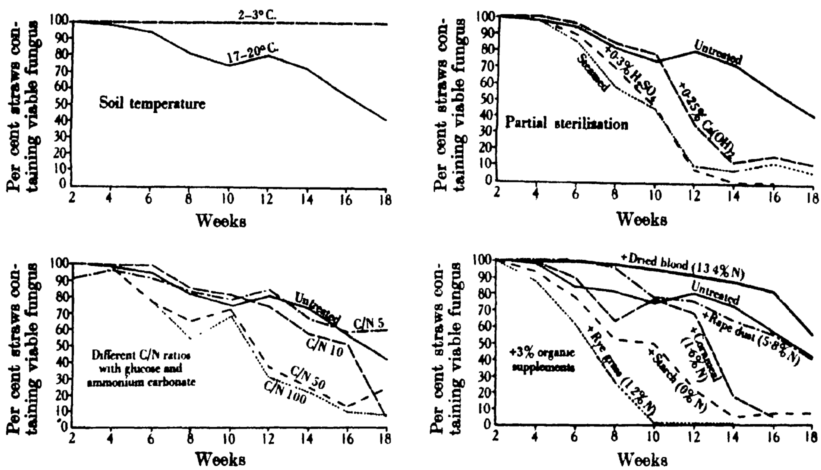
	Weeks								
Soil series	2	4	6	8	10	12	14	16	18
No. 1, Carbello	100	100	90	88	87	79	81	69	51
,, 2, Tunstall	100	98	90	78	78	64	55	34	3
,, 3, Rothamsted	100	99	83	31	13	22	5	1	0
,, 4, Woburn	100	99	88	83	57	77	43	35	16
,, 5, Slough	100	98	94	81	74	80	72	56	41
,, 6, Downham	100	92	95	93	89	80	67	51	25
,, 7, Whitchurch	100	95	88	35	22	9	12	0	1
,, 8, Aldeburgh	100	97	91	83	84	86	78	60	24
,, 9, in refrigerator	100	100	100	100	100	100	100	100	100
,, 10, +0·3% sulphuric acid	100	98	89	69	46	8	1	1	0
,, 11, +0·25% calcium hydroxide	100	100	96	84	78	36	13	17	12
,, 12, steamed and reinoculated	100	98	85	58	45	11	8	13	7
,, 13, +3% starch	100	93	78	52	49	22	5	7	7
,, 14, +3% rye-grass	100	88	61	26	1	1	1	—	—
,, 15, +3% corn-meal	100	99	89	63	76	67	17	5	0
,, 16, +3% rape dust	100	100	100	95	77	75	63	54	40
,, 17, +3% dried blood	100	99	99	97	94	90	86	80	54
,, 18, C/N 100	100	98	77	54	68	31	22	9	7
,, 19, C/N 50	100	98	77	65	72	37	25	13	24
,, 20, C/N 10	100	99	99	85	81	74	57	50	6
,, 21, C/N 5	91	96	91	83	78	84	66	58	59

(i) From an examination of rates of decline in the different field soils of series 1–8, no correlation can be made out between rate of decline and pH value of the soil (which affects so markedly the parasitic activity of *Ophiobolus*). Two soils, those of Rothamsted and Whitchurch, stand out in marked contrast to the remainder in virtue of the high rate of decline in them. These two soils are alike in being clay loams, and the heaviest soils in the series. It scarcely seems likely that reduced aeration in these soils can be the reason for the rapid loss of *Ophiobolus* viability in them, since improved aeration seemed to increase rate of decline in the flower-pots of Exp. I, whilst in Exp. III this is confirmed; it will, further, be shown that *Ophiobolus* declines more slowly in a waterlogged soil than in one at medium moisture content. From the results of other workers (Waksman, 1931), it might be expected that numbers of bacteria and

actinomycetes would be considerably higher in these two heavy soils than in the six remaining soils.

(ii) No loss of viability was revealed by the test throughout the duration of the experiment in the series kept at 2–3° C. in the refrigerator.

(iii) A marked increase in rate of decline of viability occurred as a result of pre-treatment of Slough soil with sulphuric acid and with lime, as in Exp. I, and also as a result of partial sterilization by steam and subsequent reinoculation of the soil. These results, together with the apparent absence of any direct relation between soil *p*H and rate of decline in series 1–8 of this experiment, suggest that the action of the chemicals has



Text-fig. 2. Decline in viability of the fungus in the soils of Exp. II.

produced a partial-sterilization effect (Hutchinson & MacLennan, 1914), similar to that of steaming, and that the enhanced power of these three soils to decompose *Ophiobolus* mycelium is due to the sudden rapid increase in microbiological activity as a result of partial sterilization.

(iv). The effectiveness of the organic supplements added to the soil in series 13–17 in accelerating decline of *Ophiobolus* appeared to fall off with increasing nitrogen content above 1·2% of dry matter (rye-grass meal). In Table IV the nitrogen contents of the different organic supplements are set out against the approximate times for 50% decrease in viability of the fungus in these series. It will be observed that rape dust produced no acceleration and dried blood a retardation in decline of the fungus.

Table IV. *Nitrogen contents of organic supplements in relation to the viability of* Ophiobolus

	Starch	Rye-grass	Corn-meal	Rape dust	Dried blood	No treatment
Time in weeks to 50% decrease in viability of fungus	9	6	13	17	18+	17
Nitrogen content of added organic matter as % dry matter	0	1·2	1·6	5·8	13·4	—

(v) It is unfortunate that a glucose alone treatment was not included in series 18–21 with variable C/N ratio. The most rapid decline of the fungus occurred in series 18 with C/N ratio 100, and the effectiveness of the glucose appeared to be progressively reduced by increasing amounts of nitrogen up to a C/N ratio of 5. In this series, decline was no more rapid than in the untreated control soil, and there is indeed evidence of an actual retardation due to the treatment at 18 weeks.

Exp. III. The contrast observed in the previous experiment between the action of rye-grass meal and that of dried blood seemed so striking as to merit further investigation. In this experiment, accordingly, four rather different soils were selected to receive dressings of rye-grass meal at three different levels, and of dried blood at two different levels. All soils were started and maintained at a moisture content of 50% saturation.

The rye-grass meal was applied at the rates of 6, 1 and 0·2 g. per tumbler, and the dried blood at the rates of 1 and 0·2 g. per tumbler. These amounts were added per 180 c.c. of dry soil to the different soils, since owing to their different "apparent densities" (Robinson, 1936), volume seemed to afford a better basis of equivalence than weight. Since dried blood is very rapidly decomposed in the soil, it seemed better to omit the dressing of 6 g. per tumbler, as likely to lead to excessive accumulations of soluble substances which might prove toxic to the *Ophiobolus* mycelium, and produce misleading results.

The four soils employed were as follows:

Bridgham: a very light sandy soil of the "Breckland" type from Norfolk, containing some 7% of calcium carbonate, and giving a *p*H value of 7·8.

Woburn: a typical light loam from the Woburn Experiment Station, of *p*H 6·4.

Downham: a black fen soil from Norfolk, of *p*H 7·8.

Slough: as used in previous experiments but this batch of soil was somewhat acid, giving a *p*H value of 6·2.

The drift in *p*H values in the differently treated soils was followed by means of the quinhydrone electrode (Table V).

Table V. *p*H *values of soils from Experiment III*

Soil series	Weeks 2	4	6	8	10	14
Series 1–6, Bridgham:*						
No. 1, untreated	7·8	7·8	7·9	—	—	—
,, 2, +0·2 g. rye-grass	7·8	7·9	7·9	—	—	—
,, 3, +1 g. rye-grass	7·8	7·9	7·9	—	—	—
,, 4, +6 g. rye-grass	7·9	8·1	8·1	—	—	—
,, 5, +0·2 g. dried blood	7·5	7·6	7·9	7·6	7·5	7·9
,, 6, +1 g. dried blood	7·3	7·4	7·5	7·5	7·4	7·6
Series 7–12, Woburn:						
No. 7, untreated	6·4	6·3	6·4	6·6	6·4	6·6
,, 8, +0·2 g. rye-grass	6·2	6·3	6·2	6·5	6·4	6·6
,, 9, +1 g. rye-grass	6·4	6·5	6·4	6·5	6·5	6·9
,, 10, +6 g. rye-grass	6·6	6·6	6·8	6·6	6·7	7·1
,, 11, +0·2 g. dried blood	5·7	5·8	6·0	6·2	6·1	6·6
,, 12, +1 g. dried blood	6·0	4·9	4·6	4·6	4·5	4·8
Series 13–18, Downham:						
No. 13, untreated	7·5	7·7	7·8	7·8	7·8	7·8
,, 14, +0·2 g. rye-grass	7·5	7·7	7·8	7·8	7·8	7·8
,, 15, +1 g. rye-grass	7·6	7·8	7·7	7·9	7·8	7·8
,, 16, +6 g. rye-grass	7·7	7·8	7·8	7·9	7·8	7·9
,, 17, +0·2 g. dried blood	7·3	7·5	7·6	7·6	7·6	7·7
,, 18, +1 g. dried blood	7·1	7·2	7·3	7·5	7·4	7·5
Series 19–24, Slough:						
No. 19, untreated	6·1	6·2	6·2	6·3	6·3	6·6
,, 20, +0·2 g. rye-grass	6·2	6·2	6·0	6·4	6·0	6·5
,, 21, +1 g. rye-grass	6·2	6·4	6·4	6·1	6·1	6·5
,, 22, +6 g. rye-grass	6·6	6·5	6·3	6·3	6·4	6·7
,, 23, +0·2 g. dried blood	5·7	5·9	5·8	5·8	5·9	6·5
,, 24, +1 g. dried blood	6·0	5·2	4·5	4·5	4·6	4·8

* Quinhydrone electrode determinations with the Bridgham soil gave *p*H values showing drift in the alkaline direction attributable to manganese error (Crowther & Heintze, 1930). *p*H determinations of the Bridgham soils are, therefore, only approximate.

As a subsidiary experiment, a parallel series with Slough soil at 50% saturation was set up in 4 in. flower pots as in Exp. I, but here the sand mulch was omitted, further to improve soil aeration. A second batch of Slough soil was waterlogged in tumblers, and maintained at 100% saturation throughout the experiment.

The complete results of this experiment are set out in Table VI, and are illustrated in Text-fig. 3.

The following conclusions may be drawn:

(i) The decline of the fungus was considerably more rapid in the two better soils, Slough and Downham, than in the two poorer soils, Woburn and Bridgham.

Table VI. *Percentage viability of* Ophiobolus *resting mycelium in infected straws*

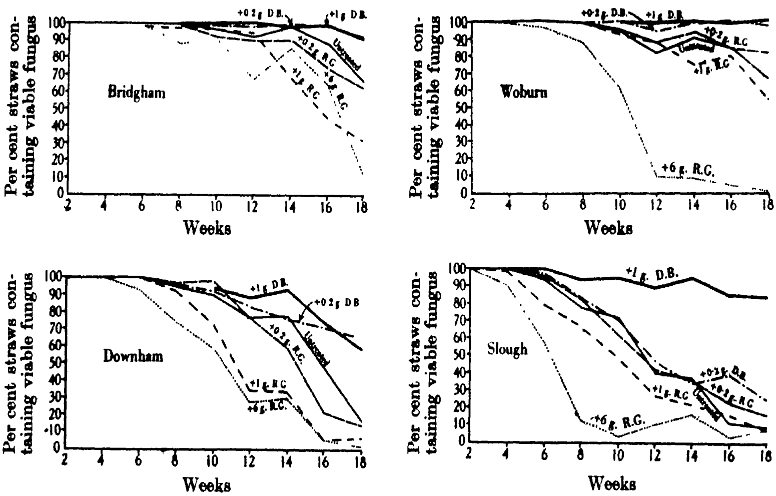
Soil series	Weeks 2	4	6	8	10	12	14	16	18
Series 1–6, Bridgham:									
No. 1, untreated.	100	100	100	100	96	92	98	88	66
,, 2, +0·2 g. rye-grass	100	100	100	99	92	90	90	74	62
,, 3, +1 g. rye-grass	100	100	98	97	96	94	68	45	31
,, 4, +6 g. rye-grass	100	100	98	88	90	67	85	64	12
,, 5, +0·2 g. dried blood	100	100	100	100	98	98	99	98	92
,, 6, +1 g. dried blood	100	100	97	99	100	100	98	99	91
Series 7–12, Woburn:									
No. 7, untreated	100	100	100	99	94	81	90	84	66
,, 8, +0·2 g. rye-grass	100	100	98	100	95	87	93	84	81
,, 9, +1 g. rye-grass	100	100	100	99	92	87	74	79	54
,, 10, +6 g. rye-grass	100	99	96	87	60	9	8	4	1
,, 11, +0·2 g. dried blood	100	100	100	99	100	94	99	100	97
,, 12, +1 g. dried blood	100	100	100	99	100	99	100	99	100
Series 13–18, Downham:									
No. 13, untreated	100	100	100	95	90	77	78	48	17
,, 14, +0·2 g. rye-grass	100	100	100	97	98	77	59	22	14
,, 15, +1 g. rye-grass	100	100	100	92	73	35	34	6	7
,, 16, +6 g. rye-grass	100	100	93	74	59	28	30	6	2
,, 17, +0·2 g. dried blood	100	100	98	96	92	83	76	71	65
,, 18, +1 g. dried blood	100	100	100	94	94	88	93	74	58
Series 19–26, Slough:									
,, 19, untreated	100	100	94	78	72	40	37	11	8
,, 20, +0·2 g. rye-grass	100	100	96	83	63	42	36	23	16
,, 21, +1 g. rye-grass	100	99	80	67	49	27	22	16	7
,, 22, +6 g. rye-grass	100	91	58	13	3	10	16	3	7
,, 23, +0·2 g. dried blood	100	100	98	84	71	47	35	39	25
,, 24, +1 g. dried blood	100	100	100	94	95	89	95	85	84
,, 25, soil in flower-pots	100	100	97	66	24	7	5	8	—
,, 26, waterlogged soil	100	100	100	100	85	64	58	28	32

(ii) Increasing dressings of rye-grass meal produced a progressively greater effect on the decline of the fungus, but the treated soils still fell into the same order of effectiveness at all levels of treatment with rye-grass meal as the untreated soils. There was one exception caused by the unexpectedly good response of Woburn soil to the 6 g. dressing; this soil was then slightly more effective than Downham soil with the same dressing.

(iii) Both the 1 g. and the 0·2 g. dressings of dried blood retarded decline of the fungus in all four soils. The effect was most striking in the case of the 1 g. dressing with Slough soil; the 0·2 g. dressing with this soil only appeared to exert a significant effect at the two last samplings.

The poor performance of the Bridgham soil is of particular interest inasmuch as severe outbreaks of the take-all disease in crops of wheat and barley were noted during the 1937 season on land of this type that had been under sugar beet as the previous crop (Garrett, 1938*b*).

The results of the subsidiary experiment on the effect of increased aeration in 4 in. flower-pots, on the one hand, and of waterlogging, on the other, are also given in Table VI. Decline in viability of *Ophiobolus* was considerably more rapid in the flower-pots in this experiment than it was in Exp. I. The greater difference between the flower-pot series and the tumbler controls in this experiment may be partly attributed to the fact that no sand mulch was added to the flower-pots, in an endeavour still further to increase aeration. This led to more rapid drying of the soil between waterings, which were made at intervals of 3–4 days. The possibility that the rapid fluctuation in soil moisture content adversely



Text-fig. 3. Decline in viability of the fungus in four soil types of Exp. III, treated with rye-grass (R.G.) and with dried blood (D.B.), respectively.

affected the resistance of the fungus mycelium cannot be excluded; at the same time, such fluctuation would result in still further improvement in soil aeration, which would be reflected by increased microbiological activity. Fluctuating soil conditions are known to be more conducive to the microbiological efficiency of the soil as a whole (Waksman, 1931) than are very uniform and constant conditions, such as are obtained in an incubator (Exp. I). The fact that the viability of *Ophiobolus* declined more slowly in the waterlogged soil than in the soil at 50% saturation again indicates that the resting mycelium of the fungus is more tolerant of adverse physical conditions of the soil than it is of conditions favouring general microbiological activity.

In order to supplement the conclusions derived from Exp. III, a series of observations on the microflora developing in the differently treated soils was made by means of the *soil plate* method, originally devised by Cholodny (1930) for direct microscopical study of the soil flora. This method has already been applied with success by King *et al.* (1934) to a study of the biological control of *Phymatotrichum omnivorum*, causing cotton-root rot, by means of organic manures. In this experiment, the four soils Slough, Downham, Bridgham and Woburn were compared (*a*) untreated, (*b*) with addition of the 1 g. dressing of dried blood per tumbler, (*c*) with addition of the 1 g. dressing of rye-grass meal per tumbler. Tumblers were set up in duplicate; two clean microscope slides were buried vertically, some 3 cm. apart, in the dry soil of each tumbler before adding the correct amount of distilled water to bring the soil to 50 % saturation. The slides were removed for examination at 10, 14 and 28 days. The balls of moist soil, containing the slides, were knocked smartly out of the tumblers; the slides were then pulled away from each face of soil in turn, as recommended by Conn (1932). After removal from the soil, the slides were air-dried and one side was cleaned with a pad of damp cotton wool. The slides were then immersed for 1 min. in the boiling staining and fixing solution, consisting of 5 % erythrosin in 5 % aqueous phenol, rinsed in distilled water, and air-dried. They were then stored dry, for examination at leisure under a water mount.

The Cholodny soil plate method, as originally devised, had the acknowledged disadvantages, when compared with the agar plate counting methods, of being only a qualitative method, and of offering little opportunity for the identification of the observed micro-organisms even as genera. In order to overcome the first of these two disadvantages, Jensen (1934–6) devised a method of estimating the frequency of fungus mycelium on the slides, by recording the presence or absence of hyphae in 500–550 randomized microscope fields on each Cholodny slide. He employed as his microbiological quadrat a square field of 65 μ side-length, examined under a $\frac{1}{12}$ immersion objective. His method has been modified for this experiment, by restricting examination to 50 microscope fields per slide; a circular field of approximately 315 μ diameter, obtained by use of a $\frac{1}{6}$ objective and 10 × eyepiece with a body length of 200 mm., was selected; the fifty fields were examined at intervals of 1 mm. in a median longitudinal transect of the slide. The presence or absence of (*a*) fungal hyphae, (*b*) actinomycete hyphae has been recorded for each of the fifty fields examined on each slide. Since the 10-day series gave the best development of micro-organisms on the slides, this series was the

one selected for recording; the 14 and 28-day series were more rapidly examined without quantitative recording. The data given in Table VII are based on the examination of four slides from two tumblers of each soil; the following conclusions may be drawn:

(i) Only poor development of fungus and actinomycete mycelium occurred in the untreated soils, but a considerable increase in both followed the addition either of rye-grass meal or of dried blood. In the untreated soils, the best development of fungi and actinomycetes occurred in Slough soil, followed in order by that in Bridgham, Downham, and Woburn soils.

Table VII. *Percentage microscope fields showing* (*a*) *fungus mycelium and* (*b*) *actinomycete mycelium on the Cholodny slides, after a period of* 10 *days*

	Percentage occurrence of fungi			Percentage occurrence of actinomycetes		
	Untreated	+ rye-grass	+ dried blood	Untreated	+ rye-grass	+ dried blood
Slough soil	10	60	56	15	49	53
Downham soil	2	29	18	7	25	45
Bridgham soil	6	39	24	11	19	40
Woburn soil	1	25	33	2	9	22
Standard errors:						
Slough soil	±6·3	±4·2	±5·5	±2·6	±0·5	±3·8
Downham soil	±2·0	±2·4	±2·2	±0·7	±2·9	±5·7
Bridgham soil	±1·2	±7·1	±1·8	±0·7	±4·2	±6·8
Woburn soil	±0·7	±1·6	±7·6	±2·0	±3·1	±6·8

(ii) In the treated soils, the fungi developed best in Slough soil, occurring in some 60% of the fields on eight slides; in the Bridgham and Woburn soils, fungi occurred in some 30% of the fields, whilst in the Downham soil, they occurred only in some 25% of fields.

(iii) In the treated soils, the actinomycetes developed best in Slough soil, occurring in some 50% of the fields; they occurred in 35% of the fields in Downham soil, in 30% of the fields in Bridgham soil, and in only 15% of the fields in Woburn soil.

(iv) Rye-grass appears to have been scarcely more effective than dried blood in promoting fungus development; thus rye-grass was more effective than dried blood in Downham and Bridgham soils, approximately equally as effective in Slough soil, and actually less effective in Woburn soil.

(v) Dried blood, on the other hand, was definitely more effective than rye-grass in promoting actinomycete development; actinomycete mycelium was approximately twice as abundant on the dried blood slides

as on the rye-grass slides in the Downham, Bridgham and Woburn soils; in Slough soil there was no significant difference.

The observations as a whole indicate greatest microbiological activity in the Slough soil, in which, also, *Ophiobolus* disappears most rapidly. They have failed to indicate any differences in the development of fungi and actinomycetes which might explain the diverse effects of rye-grass meal and of dried blood on the rate of decline of *Ophiobolus*; the development of fungi was almost as good, and that of the actinomycetes much better, in the dried blood soils as in the rye-grass soils.

Examination of the slides left in the soils for 14 and for 28 days, respectively, supported the observations of other investigators cited above as to the rapid development and decline of the original soil microflora in the treated soils. The relationship of bacteria to the disappearing fungus hyphae was especially noticeable on the 14-day and 28-day slides; former sites of fungus hyphae were frequently marked out by the almost continuous development of small bacterial colonies. The part played by bacteria in the decomposition of fungus hyphae is especially well demonstrated by this technique.

Exp. IV. In this experiment, infected straws were buried in soil at the standard rate of 50 straws per 200 g. (=180 c.c.) of air-dry soil, and also at the rates of 10 and 200 straws, respectively, per 200 g. of soil. The "200" straw/soil ratio was arranged by burying 100 straws in 100 g. of air-dry soil per tumbler, since 100 straws has been the standard sample throughout these experiments. In this series, the straws were placed in contact, and there was then enough dry soil to fill up all the interstices and provide for a covering of soil some 1 cm. deep on top. A sample of 100 straws from each series was taken every 3 weeks to 21 weeks, instead of at the usual fortnightly intervals. The effect of the straw/soil ratio on the decline of *Ophiobolus* is shown by the results of this experiment, given in Table VIII.

Table VIII. *Percentage viability of* Ophiobolus *resting mycelium in infected straws*

	Weeks						
Series	3	6	9	12	15	18	21
No. 1, 10 straws per 200 g. soil	100	100	99	96	96	88	89
,, 2, 50 straws per 200 g. soil	100	99	99	98	91	91	84
,, 3, 200 straws per 200 g. soil	100	99	95	83	51	43	36

The more rapid decline of *Ophiobolus* in the "200" series than in the "10" and "50" series may be attributed partly, no doubt, to improved aeration of the soil by incorporation of the additional straw. It is also

possible that by increasing the number of straws, the number of organisms stimulated into activity in the soil immediately around each individual straw is also increased. Both of these factors, however, may be subordinate to a third—that of nitrogen poverty of the soil induced by crowding of the straws.

Exp. V. No experiments on the rate of dying out of *Ophiobolus* from the infected straws under sterile conditions have been performed, owing to the technical difficulties of operating with sterilized soil and effectively maintaining sterile conditions over the long periods likely to be required. A test has been made, however, of the viability of the fungus in 100 infected straws kept for a period of one year in the original culture flask in the open laboratory, i.e. under conditions of incubation precisely similar to those of the experiments with soil in tumblers. The fungus was found to be alive in all the straws, causing vigorous infection of every wheat seedling. These tests will be continued.

IV. Discussion

The results of these experiments seem to justify the thesis put forward in the introduction to this paper—that decline in the viability of the resting mycelium of *Ophiobolus* in infected wheat straw is due primarily to its natural decomposition by the other soil micro-organisms. Decline of viability in *Ophiobolus* can be correlated with microbiological activity of the soil throughout the experiments. Thus, decline is indefinitely postponed by absence of moisture in air-dry soil (Exp. I), by low temperature (Exp. II), and by the exclusion of other micro-organisms (Exp. V); it is retarded by the continued incubation of the soil under uniform conditions in an enclosed space (Exp. I). Decline is also retarded for a time under the anaerobic conditions of a waterlogged soil, so that the fungus actually disappears more quickly in soil at 50% saturation than in that at 100% saturation (Exp. III).

Decline of the fungus is accelerated, on the other hand, by addition to the soil of soluble or finely divided organic materials relatively poor or lacking in nitrogen, such as glucose, starch and rye-grass meal, which promote a rapid temporary increase in numbers and activity of the soil micro-organisms (Exps. I–III). The marked influence of pre-treating the soil with steam, lime, and sulphuric acid in accelerating decline may be attributed tentatively to the partial sterilization effect, resulting in subsequently increased numbers and activity of micro-organisms in the treated soils (Exps. I and II). Decline is most rapid in the two heaviest and richest soils of Exp. II, and proceeds more quickly in the two better

soils of Exp. III than in the two poorer soils. It is considerably more rapid in Slough soil than in inoculated sand (Exp. I), though aeration is better in the latter (improved aeration in the flower-pots of Exps. I and III produced an increase in rate of decline over that in the parallel series in tumblers). It may, therefore, be suggested that rate of decline of *Ophiobolus* is a function of the numbers and activity of micro-organisms in the soil. The rates of decline in the four soils of Exp. III seem to be related both to the initial biological state of the soil and to the amount of suitable energy material (rye-grass meal) added; this is intelligible on reflexion that numbers and activity of micro-organisms would rise more rapidly in a rich soil after addition of a given amount of energy material than those in a poor soil receiving the same dressing.

The Cholodny slides of Exp. III demonstrated the rapid development and disappearance of the original microflora resulting from the addition of 1 g. of rye-grass meal or of dried blood per tumbler to the different soils. This suggests that the rate of decline of the *Ophiobolus* mycelium in the infected straws may possibly be determined by the initial microbiological activity of the soil during the first week or two weeks of the experiment; after this period, the rate of decomposition may possibly be independent microbiologically of the surrounding soil, and affected chiefly by the non-biological conditions of incubation. It must be remembered, too, that attack proceeds from within the straw cavity as well as from the outside of the straw in these experiments.

The retarding effect apparently exercised by excess of nitrogen on the decline of *Ophiobolus* is one of the most interesting features of Exps. II and III. Both Waksman & Starkey (1923) and Winogradsky (1925) have shown that whereas substances rich in cellulose such as cereal straw and rye-grass are decomposed under aerobic conditions by fungi, actinomycetes, and bacteria, substances rich in nitrogen such as dried blood bring about much greater rises in numbers of bacteria than in those of fungi and actinomycetes. The Cholodny slides of Exp. III showed, however, nearly as great a development of fungi and twice as great a development of actinomycetes on the dried blood slides as on the rye-grass slides and, in both, the microfloral development was greatly in excess of that on slides from the untreated soils. It appears, therefore, that, under aerobic conditions, dried blood promotes a richer flora of fungi, actinomycetes and bacteria than does ground rye-grass.

More subtle differences in microfloral development according to the type of organic matter added to the soil cannot yet be excluded from consideration; it seems likely, however, that the retarding action of excess

of nitrogen on the disappearance of *Ophiobolus* may be a more direct effect. Thus an excess of nitrogen may, in some way, increase the resistance of the *Ophiobolus* resting mycelium.

A third possible explanation is suggested by the fact that fungus mycelium is itself rich in nitrogen, containing some 4–8% (Waksman, 1931); it has been successfully employed as a source of organic nitrogen in decomposition studies by Heck (1929), Jensen (1932), and Norman (1933). Since the total nitrogen content of the infected straws averaged only 0·4%, which is only about one-third the amount required for complete decomposition of normal oat straw (Norman, 1931), it may be suggested that the *Ophiobolus* mycelium itself serves as a source of nitrogen for the decomposition of the straw. The addition of organic substances rich in nitrogen, or of inorganic nitrogen in the form of ammonium carbonate as added in Exp. II may, therefore, prolong the life of *Ophiobolus* by protecting it from the nitrogen demands of the organisms engaged in decomposing the straw. This suggests that the effectiveness of rye-grass meal in promoting rapid decline of *Ophiobolus* may be due not only to increase in numbers of organisms multiplying on the added energy material but, also, to the promotion of a nitrogen scarcity in the soil around the straws. The more rapid decline of the fungus in the straws crowded at the rate of 200 per 200 g. of soil in Exp. IV may be attributed, in part at least, to the operation of this factor.

This hypothesis of nitrogen scarcity as a factor directly accelerating the disappearance of *Ophiobolus* mycelium throws a fresh light on certain observations previously difficult to explain. Throughout the experiments, it had been observed that decline of the fungus in the different series frequently failed to agree with obvious decomposition of the straw, as judged by darkening in coloration, loss of rigidity and increase in friability, etc. It had been especially remarked in Exps. I–III that straws receiving the 6 g. per tumbler dressing of rye-grass meal appeared to decompose most slowly of all, keeping their fresh appearance and rigidity the longest of any series; the same was observed of the closely crowded straws in Exp. IV. These findings, thus, exactly reverse the original expectation that the addition of a nitrogenous fertilizer, by promoting more rapid decomposition of the straw, would hasten also the disappearance of the *Ophiobolus* mycelium.

Some new problems, therefore, await solution as a result of this work. For how long after burial in the soil is the decomposition of the fungus mycelium in the infected straw dependent microbiologically on the outside soil, and how does this period vary with the individual soil? What is

the mechanism of the apparent protective effect of added nitrogen towards the *Ophiobolus* mycelium, and can this be due to preferential decomposition of a more easily available source of nitrogen? Do organic materials such as dried blood exercise an accelerating effect as energy materials for the multiplication of the soil microflora which is masked by their depressing effect as nitrogen sources? Experiments designed to answer some of these questions are now in progress. At the same time, experiments to link up these observations with the disappearance of *Ophiobolus* mycelium under more natural soil conditions are necessary. An experiment at present in progress indicates that decline of the fungus in infected straw is considerably slower under natural soil conditions outside than in the laboratory tumblers; this is to be attributed chiefly to lower soil temperatures, as loss of viability during the winter months is almost negligible.

In conclusion, reference may be made to the work of King *et al.* (1934), who have demonstrated the controlling effect of repeated applications of farmyard or green manure on the occurrence of cotton root rot, due to the fungus *Phymatotrichum omnivorum*, in Arizona. As a practical method for the control of this disease, King (1937) recommends the ploughing under of very heavy dressings of organic manures in deep furrows during autumn or early winter. One or more irrigations are given to encourage decomposition, which must also be favoured by the comparatively high soil temperatures (12–15° C.) of the Arizona winter season. In the spring, the rows of cotton are planted immediately over the buried decaying organic material. The striking reduction in the incidence of disease obtained by King on the manured plots may be attributed, in part at least, to a more rapid decomposition of the resting *Phymatotrichum* mycelium under such conditions.

V. Summary

A study has been made of the decline in viability of *Ophiobolus graminis* as resting mycelium in artificially infected wheat straw buried in the soil. The pieces of straw were buried in variously treated soils set up in glass tumblers, and were examined at intervals for the presence of still viable *Ophiobolus* mycelium by means of a wheat seedling test.

The experimental results suggest that the disappearance of *Ophiobolus* from the straws was due to natural decomposition by the other soil microorganisms since, in its resting phase, the fungus tolerated adverse physical conditions of the soil better than conditions optimum for microbiological activity. Decline in viability of the fungus appeared to be indefinitely

postponed in air-dry soil, in soil at 2–3° C., and under sterile conditions in the culture flask; it was less rapid in a waterlogged soil than in one maintained at medium moisture content. The soil conditions least favourable for the advance of *Ophiobolus* along the host roots in its parasitic phase (Garrett, 1936) may best preserve it during its resting phase because they are also unfavourable for general microbiological activity.

Loss of viability was hastened by the addition of energy materials poor or entirely lacking in nitrogen, such as glucose, starch, and rye-grass meal, to the soil; it was more rapid in a partially sterilized and reinoculated soil than in an untreated soil. These results may be attributed to the rise in numbers and activity of soil micro-organisms following upon the treatments. The rate of decline of the fungus varied with soil type, being more rapid in rich and heavy soils than in poor, light soils. Rate of decline was apparently not directly affected by soil reaction, nor appreciably by moisture content of the soil over the range 30–80 % saturation. Decline of the fungus was more rapid under conditions of fluctuating soil moisture and improved aeration in unglazed flower-pots than under more uniform conditions in glass tumblers; it was slowest in soil maintained under still and uniform conditions in a small closed incubator.

The most rapid disappearance of the *Ophiobolus* resting mycelium, therefore, seems to have been secured by conditions favouring maximum microbiological activity in the soil. The decline in viability of the fungus did not necessarily proceed parallel with gross decomposition of the infected straw as a whole. It was delayed by the addition of dried blood, containing 13 % nitrogen, to the soil, whereas this treatment accelerated decomposition of the straw. Decline of the fungus was accelerated by addition of rye-grass meal, which delayed decomposition of the straw by taking up the available nitrogen. It is suggested that the *Ophiobolus* mycelium may itself serve as a source of nitrogen for the decomposition of the straw, and that the rapidity of its disappearance may be related directly to the degree of nitrogen scarcity in the soil and straw medium.

I have much pleasure in thanking Dr A. G. Norman for various useful suggestions, and for all the nitrogen determinations. I am especially indebted to Miss L. Cunow and to Miss M. M. Browne for help in the carrying out of the experiments.

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EXPLANATION OF PLATE XXVIII

Wheat seedling test for the presence of viable *Ophiobolus* mycelium in the infected straws. On left, four straws in which the viability of *Ophiobolus* is still undiminished; on right, four straws in which the fungus is no longer viable.

(*Received* 28 *March* 1938)

GARRETT.—SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT (pp. 742–766)

THE STERILIZATION OF LETTUCE SEED

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(With Plate XXIX and 7 Text-figures)

Introduction

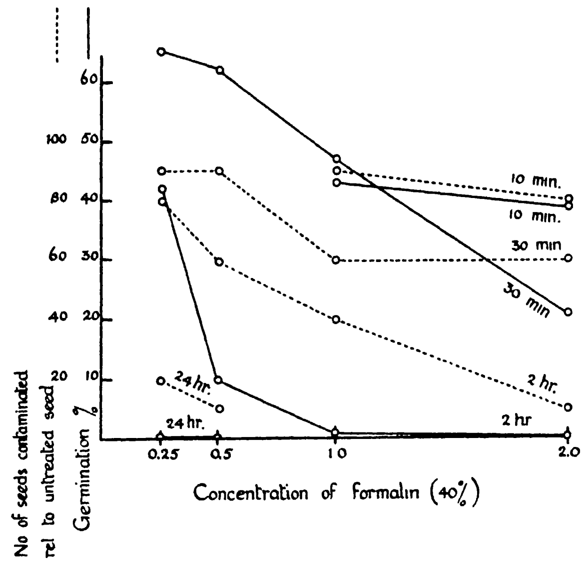
The marked development in recent years of methods of seed sterilization has been followed by attempts to control diseases—reputed to be seed borne—of lettuce seed. The application by lettuce growers of standard treatments recommended for cereals has resulted in serious damage to the seed. Similar cases of severe damage, quoted by Niethammer (1930), have been reported from Germany and Holland. Niethammer (1932) also records that lettuce seed fails to tolerate concentrations of organo-mercurial disinfectant that are innocuous to seeds of other vegetables, such as spinach and parsley. Moreover, disinfection by such a method was not efficacious in the case of lettuce seed. The comparative immunity of cereal seed to organo-mercurial chemicals has been attributed to the semi-permeable nature of the seed-coat, which absorbs relatively small amounts of mercury, whereas lettuce seed, which does not possess a semi-permeable coat, is said to absorb relatively large quantities of mercury. The present investigation was carried out in order to discover a safe and efficient method of sterilizing lettuce seed, which might be recommended to growers.

The effect of germicides at different concentrations and for different periods of treatment has been tested by (A) germinating treated seed upon a thin film of nutrient agar in sterile Petri dishes, the efficiency of the sterilization being estimated from the growth upon the agar of bacteria normally present on the seed coat, (B) sowing treated seed in sterilized soil in seed trays in order to ascertain the effect upon lettuce seed of treatments that have from time to time been recommended for the control of various seed-borne diseases.

A. Treated seed germinated in Petri dishes

The effects of various combinations of concentration of formalin and period of treatment on germination capacity and contamination of the seed-coat of the variety Golden Ball are given in Table I. These results

are represented graphically in Text-fig. 1, which shows that the slopes of the curves representing the fall in viability are steeper than those representing the fall in number of contaminated seeds. The effect of increase in potency of treatment is thus more marked upon seed injury than upon efficiency of sterilization. No treatment that allowed a high germination percentage gave satisfactory clean seed.



Text-fig. 1. The % germination of Golden Ball lettuce seed sterilized with different concentrations of formalin. The efficiency of sterilization, as estimated from relative number of seeds with viable bacteria, is also shown.

Table I. *The effect of concentration of formalin* (40 %) *and time of treatment on % germination of Golden Ball lettuce seed sown on nutrient agar. Values in brackets are relative numbers of seeds with viable bacteria on their coats. Germination of untreated seeds* = 58 %

	0·25 %	0·5 %	1·0 %	2·0 %
10 min.	—	—	43 (90)	39 (80)
30 min.	65 (90)	62 (90)	47 (60)	21 (60)
2 hr.	42 (80)	10 (60)	1 (40)	0 (10)
24 hr.	0 (20)	0 (10)	—	—

The effect on the viability of Golden Ball of all combinations of three concentrations of copper sulphate—0·5, 1·0 and 2·0 %—and three periods

of treatment—0·75, 2·0 and 4·5 hr.—is illustrated by the following results (mean of four samples with standard error):

1 % for 0·75 hr.	1 % for 4·5 hr.	2 % for 4·5 hr.
46 ± 3 %	63 ± 3 %	33 ± 4 %

A concentration of 1 % for 0·75 hr. does not affect viability (49 % for untreated seeds), whereas the same concentration for a longer period increases the percentage of germinating seeds, possibly due to the suppression of contaminating organisms on the seed coat. Although the least potent treatment of 0·5 % for 0·75 hr. reduced the number of contaminated seeds by 50 %, about 10 % of the seeds remained contaminated even after 2·0 % for 4·5 hr., a treatment which has a definite detrimental effect on viability.

B. Treated seed sown in seed trays

Over 6000 seeds were sown in samples of about fifty. The following treatments were applied to each of the varieties Trocadero, Gotte à Forcer and Golden Ball:

Dip treatments			Dust treatments
Copper sulphate	1·0 %	for 1 hr.	Tillantin R
Mercuric chloride	0·25 %	for 15 min.	Semesan
Uspulun	0·25 %	for 1 hr.	Ceresan
Formalin	0·25 %	for 30 min.	Copper carbonate
Calcium hypochlorite (to give 2 % Cl_2)		30 min.	Cuprous oxide

All the seeds treated by dips were washed in sterile water before sowing but a duplicate series (copper sulphate, mercuric chloride, and uspulun treatments) was carried out in which the seeds were dried before sowing without washing. The relative adhesiveness of the dusts was as follows: cuprous oxide = very good; Tillantin R, Semesan and Ceresan = satisfactory; copper carbonate = poor.

Table II. *The viability of lettuce seed sterilized by different methods and sown in seed trays. Treatments causing statistically significant decrease of germination are grouped below dividing lines*

Trocadero	%	Gotte à Forcer	%	Golden Ball	%
Untreated	82	Copper carbonate	79	Untreated	92
Cuprous oxide	82	Untreated	78	Copper carbonate	92
Copper sulphate	80	Copper sulphate	78	Uspulun	92
Semesan	79	Uspulun	77	Copper sulphate	90
Copper carbonate	78	Tillantin R	76	Formalin	89
Formalin	77	Cuprous oxide	75	Cuprous oxide	88
Uspulun	76	Calcium hypochlorite	72		
Calcium hypochlorite	75			Calcium hypochlorite	85
		Formalin	71	Semesan	75
Tillantin R	51	Semesan	67	Tillantin R	37
Mercuric chloride	2	Mercuric chloride	51	Mercuric chloride	1
Ceresan	0	Ceresan	8	Ceresan	1

The effect of the treatments on germination is shown in Table II, reductions that are statistically significant being separated by a line from those that are not. Injury less severe than that necessary to kill the seed is shown by retardation of germination, and many treatments that did not show final differences would have done so had an earlier count been considered. By plotting the daily stands the approximate extent of retardation may be estimated and is shown in Table III, the greatest number of untreated seeds germinating on the fourth day from sowing.

Table III. *Number of days retardation in germination of lettuce seed sown in seed trays following sterilization by different methods. The greatest number of untreated seeds germinated on the fourth day after sowing*

Trocadero		Gotte à Forcer		Golden Ball	
Cuprous oxide	5	Cuprous oxide	6	Cuprous oxide	4
Copper sulphate	5	Copper sulphate	0	Copper sulphate	5
Copper carbonate	0	Copper carbonate	0	Copper carbonate	0
Formalin	5	Formalin	5	Formalin	4
Uspulun	1	Uspulun	0	Uspulun	5
Calcium hypochlorite	0	Calcium hypochlorite	0	Calcium hypochlorite	0

From this experiment the following conclusions are drawn:

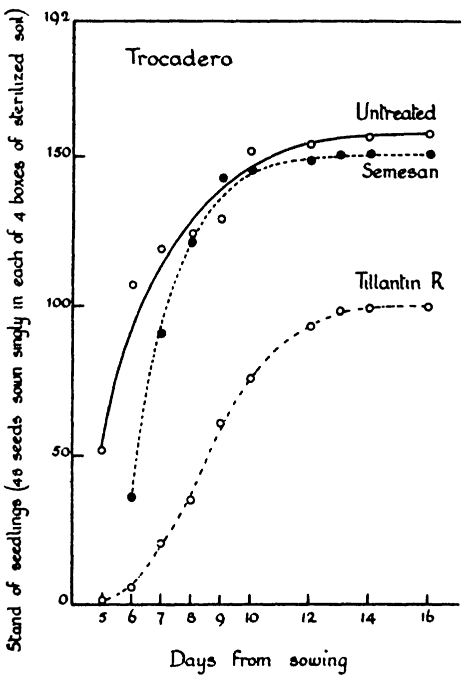
(1) With the exception of copper carbonate all the treatments were responsible on one variety or another for either a reduction in germination percentage or retardation of germination. The poor adhesiveness of copper carbonate suggests that its germicidal value is low. Since the concentrations of the dips used were weak (with the exception of mercuric chloride) it is evident that lettuce seed is abnormally sensitive and successful sterilization presents a more difficult problem than that of sterilizing cereal seed.

(2) Treatment with mercury was followed by severe seed injury. Mercuric chloride at the strength used was responsible for high mortality. Even where uspulun did not reduce the germination percentage an appreciable number of seedlings showed the deep green transparent cotyledons and blindness of the growing point symptomatic of mercury poisoning. The group of organo-mercurial dusts proved so toxic that their use for lettuce seed cannot be recommended.

(3) Appreciable reduction in the extent of retardation from copper sulphate and uspulun treatments followed the washing of the seed in sterile water before planting.

(4) The variety Gotte à Forcer is in general more resistant to seed injury than Trocadero or Golden Ball. 50% of Gotte seeds treated with

mercuric chloride germinated in comparison with 2% of Trocadero and 1% of Golden Ball. Copper sulphate and uspulun at strengths which severely retarded the germination of Golden Ball were innocuous to Gotte à Forcer. Resistance to seed injury in lettuce is a varietal characteristic.



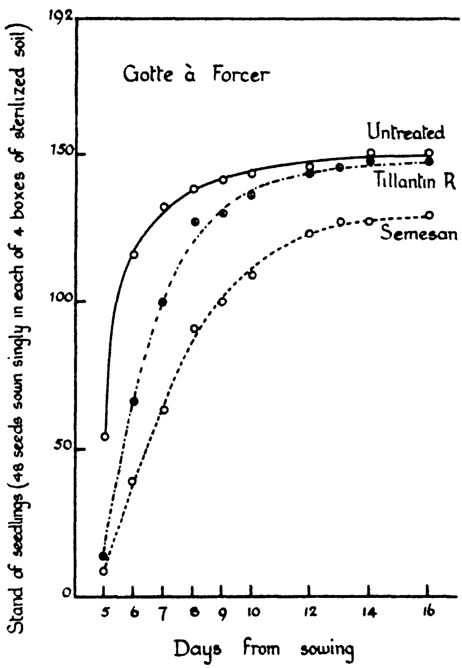
Text-fig. 2. Stand of seedlings (192 sown) of Trocadero, untreated and treated with Semesan and Tillantin R dusts (applied by shaking standard volume with measured quantity of seed in a mechanical shaker for standard period). The curves should be compared with those for Gotte à Forcer and Golden Ball in Text-figs. 3 and 4.

The differential effect of seed treatment on the germination capacity of lettuce varieties is illustrated in Text-figs. 2–4, which show the relative effects of Tillantin R and Semesan on the stands of Trocadero, Gotte à Forcer and Golden Ball respectively. The transition from the sigmoid curves characteristic of germination of injured samples and the decrement curves characteristic of the germination of untreated samples may be noted.

TRIALS WITH CALCIUM HYPOCHLORITE

From the results of the preliminary trials calcium hypochlorite was selected as the most promising treatment on the following grounds:

(1) Only a small percentage—less than 7 %—of seeds so treated had failed to germinate in comparison with untreated seeds. (Subsequent

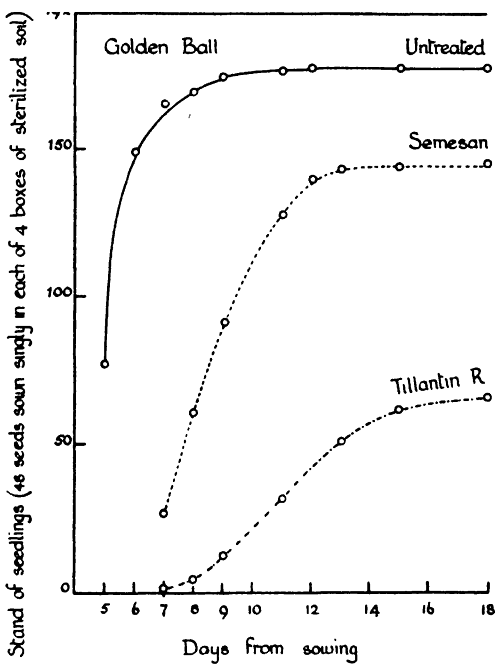


Text-fig. 3. Stand of seedlings (192 sown) of Gotte à Forcer, untreated and treated with Semesan and Tillantin R dusts (applied by shaking standard volume with measured quantity of seed in a mechanical shaker for standard period). The curves should be compared with those for Trocadero and Golden Ball in Text-figs. 2 and 4.

tests support the view that it was only the seeds already mechanically damaged that were affected.)

(2) The germination of treated seeds was not retarded and the seedlings all developed normally in contrast to treatments by mercury and copper preparations and formalin, which were followed by retarded germination and a percentage of abnormal seedlings clearly suffering from metal and other poisoning.

(3) A treatment that is partially gaseous is preferable in that irregularities in the seed coat may escape contact with sterilizing agents applied in the form of liquids and dusts.



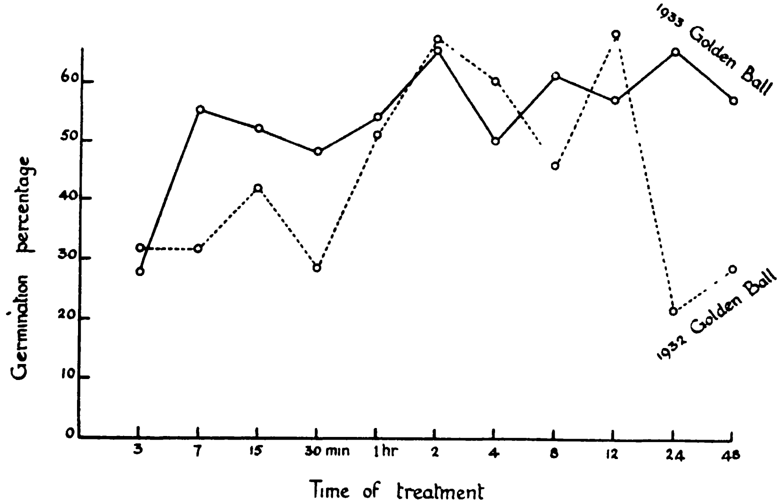
Text-fig. 4. Stand of seedlings (192 sown) of Golden Ball, untreated and treated with Semesan and Tillantin R dusts (applied by shaking standard volume with measured quantity of seed in a mechanical shaker for standard period). The curves should be compared with those for Trocadero and Gotte à Forcer in Text-figs. 2 and 3.

First set of trials, 29 *January* 1934

Triplicate samples of Golden Ball of about thirty seeds each were treated for periods ranging from 3 min. to 48 hr. and germinated in Petri dishes in contact with a thin layer of agar in order to show if the seeds were sterile. In addition to 1933 seed 1932 seed, that might be expected to possess less vigour, formed a parallel series. Table IV gives the summarized results. The germination capacity of 1932 and 1933 seed at different periods of treatment is plotted in Text-fig. 5.

Table IV. *The effect of duration of treatment with calcium hypochlorite on efficiency of sterilization and % germination of Golden Ball lettuce seed sown upon nutrient agar in Petri dishes*

Time of treatment	% germination 1932 seed	% germination 1933 seed	Number of Petri dishes (6 treated) in which all the seeds were sterile
3 min.	32	28	1
7 min.	32	55	1
15 min.	42	52	2
30 min.	29	48	3
1 hr.	51	54	4
2 hr.	67	65	6
4 hr.	60	50	3
8 hr.	46	61	4
12 hr.	68	57	4
24 hr.	22	65	4
48 hr.	29	57	4



Text-fig. 5. Germination percentage of 1932 and 1933 seed of Golden Ball (sown in Petri dishes on nutrient agar) plotted against time of sterilization with calcium hypochlorite.

The information provided by this set of trials is as follows:

(1) A sterile sample may be obtained in as short a time as 3 min. but 15 min. should be taken as the minimum time required to effect reliable sterilization (at 14° C. the temperature of treatment) since, after this period, 90% of the seeds treated were clean.

(2) An increase in the time of treatment from 3 min. to 12 hr. was associated with an increase in capacity of germination, independent of sterilization, since this was practically complete at all periods above 15 min. This rising trend of germination capacity may well be the result of an increase in supply of oxygen since, if seed is germinated on filter paper or in soil the level of germination capacity is appreciably higher (see Table V and also Text-fig. 7) and hypochlorite treatment has then little or no effect on viability. It is of interest to note that Rose (1915), Borthwick & Robbins (1928) and Thornton (1936) have demonstrated that the viability of lettuce seed may be increased by raising the level of oxygen supply.

(3) Germination was not impaired by any period of treatment save those for 24 and 48 hr. (1932 seed only). Thus, even if the maximum period tolerated is only 12 hr., ample margin of safety is provided by the method since reliable sterilization was affected in as short a time as 15 min.

Second set of trials, 12 *February* 1934

Triplicate samples of Golden Ball (1932 and 1933 seed) were treated for periods ranging from 15 min. to 96 hr. As the previous trial had shown sterilization to be satisfactory after 15 min., and it was considered possible that the agar might interfere with germination, the seed was germinated upon moist filter paper. Very good germination was obtained, that of the untreated samples being 89% (1933 seed) and 90% (1932 seed). Thus the final germination capacity of 1932 and 1933 seed does not differ but the greater vigour of the 1933 seed was shown by earlier germination, counts at the end of 24 hr. giving the following values:

1932 seed 43 ± 4% germination. 1933 seed 55 ± 2% germination.

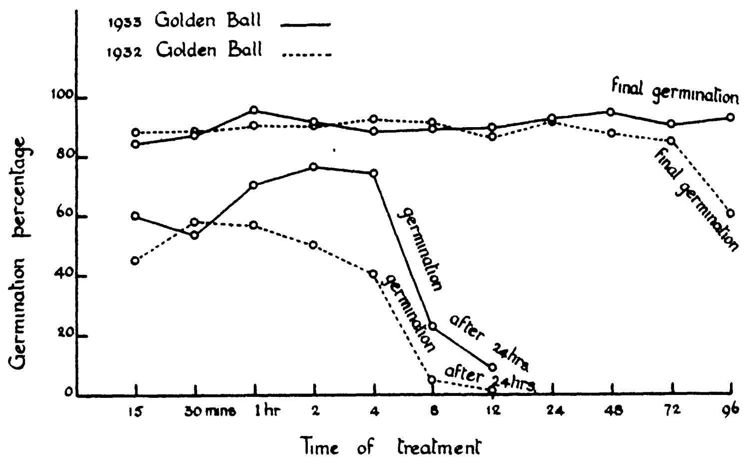
Table V. *The effect of duration of treatment with calcium hypochlorite on the germination capacity of Golden Ball lettuce seed sown upon moist filter paper in Petri dishes*

Time of treatment	1st day's germination		Final germination	
	1932 seed	1933 seed	1932 seed	1933 seed
15 min.	45	60	88	84
30 min.	58	54	88	87
1 hr.	57	70	90	95
2 hr.	50	76	90	91
4 hr.	40	74	92	88
8 hr.	5	23	91	89
12 hr.	1	9	86	89
24 hr.	—	—	91	92
48 hr.	—	—	87	94
72 hr.	—	—	84	90
96 hr.	—	—	60	92

The effect of the treatments on germination capacity are shown in Table V and Text-fig. 6, from which the following conclusions are drawn:

(1) The final germination percentage of 1933 seed has been unaffected by treatments for as long a period as 4 days, that of 1932 seed, of less vigour, for 1 or possibly 2 days.

(2) A retarding effect upon germination is apparent for periods above 4 hr. (1933 seed) and 1 hr. (1932 seed).



Text-fig. 6. Germination % of 1932 and 1933 seed of Golden Ball (sown in Petri dishes on moist filter paper) plotted against time of sterilization with calcium hypochlorite.

With a weaker solution of hypochlorite (allowed to stand for 4 hr. before use) germination was not retarded after periods of treatment less than 48 hr. (1933 seed) and was even accelerated, for the initial days germination after treatment for this period was 84% (88, 83, 82) for treated seed in comparison with 55% (57, 51, 58) for untreated seed. In contrast the germination of the 1932 seed was retarded after treatment for the same period, the values for the initial days germination being 23% (38, 20, 11) against 43% (51, 43, 36) for untreated seed.

Sterilization clearly has a differential effect upon seed samples of the same varietal constitution but differing in vigour. Treatments that leave unharmed seed of strong constitution may be unsafe to apply to older seed of less vigour.

Third set of trials, 11 *April* 1934

In these trials 1933 seed treated for periods ranging from 3 min. to 72 hr. was divided into two parallel series, one being germinated on filter paper and the other sown in the usual commercial manner in trays. In order to eliminate any possible differential effect of soaking prior to sowing the unsterilized seed, samples were soaked in glass-distilled water for periods corresponding with those of the sterilization treatment. The results are shown in Table VI.

Table VI. *The effect of duration of treatment with calcium hypochlorite on the % germination of Golden Ball lettuce seed sown on moist filter paper in Petri dishes and in sterilized soil in seed trays*

	Seed germinated in Petri dishes				Seed sown in trays			
	Examination after 1 day		Final examination		Examination after 4 days		Final examination	
Time of treatment	Steril-ized	Soaked	Steril-ized	Soaked	Steril-ized	Soaked	Steril-ized	Soaked
3 min.	49	49	85	91	39	30	84	85
7 min.	58	47	85	95	47	34	91	77
15 min.	51	48	87	88	44	38	89	71
30 min.	55	47	87	86	20	26	85	83
1 hr.	46	42	85	87	25	36	86	86
2 hr.	47	45	89	92	31	27	96	78
4 hr.	48	27	81	81	33	31	96	80
8 hr.	9	7	90	84	29	19	89	90
12 hr.	—	—	87	90	31	6	96	88
24 hr.	—	—	90	91	—	—	93	85
48 hr.	—	—	83	90	—	—	78	89
72 hr.		—	77	77	—	—	76	96

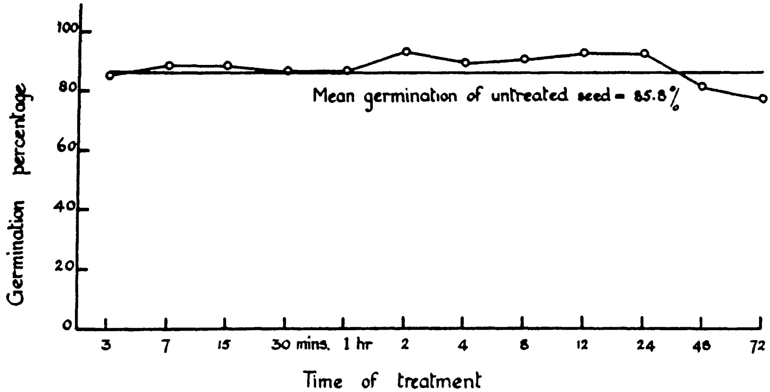
SEED GERMINATED IN PETRI DISHES

The mean germination percentage of the sterilized samples 86·3% compares favourably with the mean 88·6% of the soaked samples, and demonstrates that the treatment is harmless though applied for periods many times as long as that necessary for efficient sterilization. The values in Table VI for germination capacity suggest a tendency for earlier germination of the treated seed.

SEED SOWN IN SEED TRAYS

The relation between the sterilized and untreated but soaked samples remained the same as in Petri dishes. For seven out of nine periods of treatment the percentage germination of the sterilized seed was higher, 4 days after sowing, than that of the untreated but soaked seed. This stimulating effect of hypochlorite treatment cannot be correlated with

germicidal action, which is presumably fully exerted after the shorter periods of treatment but is probably attributable, as in the case of viability, to increase of oxygen supply. The final percentage germination of seed sown in trays by a similar method to that used by growers was not reduced below that of the untreated seed save after long periods of treatment of 48 and 72 hr. The effect on germination percentage of period of sterilization may be seen in Text-fig. 7 in which are combined the results obtained by sowing on filter paper in Petri dishes and in soil in seed trays.



Text-fig. 7. Germination % of Golden Ball (sown in Petri dishes and in seed trays) plotted against time of sterilization with calcium hypochlorite. The mean germination percentage of samples unsterilized but soaked in water prior to sowing is 85·8.

Method recommended for the sterilization by growers of lettuce seed

The method used, believed to have been originally introduced by Wilson (1915), consists of adding calcium hypochlorite ("bleaching powder") to distilled water at the rate of 5 g. per 70 c.c. (or roughly $\frac{3}{4}$ lb. per gal.), stirring, leaving for 5 min. to settle, decanting and using immediately. The original volume should be calculated so as to allow of the rejection of a third of the liquid during decantation, sufficient being left to just cover the seed. The latter may be placed in a glass bottle, which can be corked and shaken periodically to overcome the tendency of the seed to float on the surface. 4–8 hr. is recommended as a suitable period but any period between 15 min. and 24 hr. should give satisfactory results. Below 15 min. sterilization may be incomplete and above 24 hr. there is risk of seed injury. Washing the seed after treatment reduces the

risk of seed injury. Preparations of bleaching powder differ markedly in their chemical activity and the sterilization of large quantities of seed should be prefaced by observation of its effect on a small sample, which may be germinated on moist blotting paper.

SUMMARY

1. Since severe damage has followed the application by growers to lettuce seed of standard methods of sterilization recommended for cereals, trials of different methods of sterilizing lettuce seed have been carried out. Satisfactory sterilization free from seed injury is not effected by copper sulphate or formalin, while preparations containing mercury such as organo-mercurial dusts are highly toxic to lettuce seed. Sterilization with calcium hypochlorite is a safe and efficient method.

2. Treatment with calcium hypochlorite prior to sowing has an accelerating effect, independent of germicidal action, upon the germination of lettuce seed. The viability of seed sown on filter paper or in soil is not appreciably affected but that of seed sown in contact with a film of agar, which shows exceptionally poor capacity to germinate, is notably increased. These effects are not attributable to germicidal activity and possibly result from increase of oxygen supply.

3. Susceptibility of lettuce seed to injury from germicides is a varietal characteristic.

4. Fresh seed with strong viability is more resistant to injury from germicides than older seed of less vigour.

Grateful acknowledgement is due to Dr G. C. Ainsworth for taking the photographs in Plate XXIX and for reading and criticizing the MSS.

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EXPLANATION OF PLATE XXIX

Fig. 1. The toxic effect of an organo-mercurial disinfectant upon lettuce seed. The seeds sown in the box "*a*" were treated prior to sowing with cuprous oxide dust, whereas those in boxes "*b*" and "*c*" were treated with a proprietary organo-mercurial dust.

Fig. 2. Varietal resistance to injury from seed disinfectant. The seedlings in box "*a*" are the relatively resistant variety Gotte à Forcer and, in boxes "*b*" and "*c*" respectively, the relatively susceptible varieties Trocadero and Golden Ball. All seeds have been dipped prior to sowing in the same concentration of mercuric chloride for the same period.

(*Received* 21 *March* 1938)

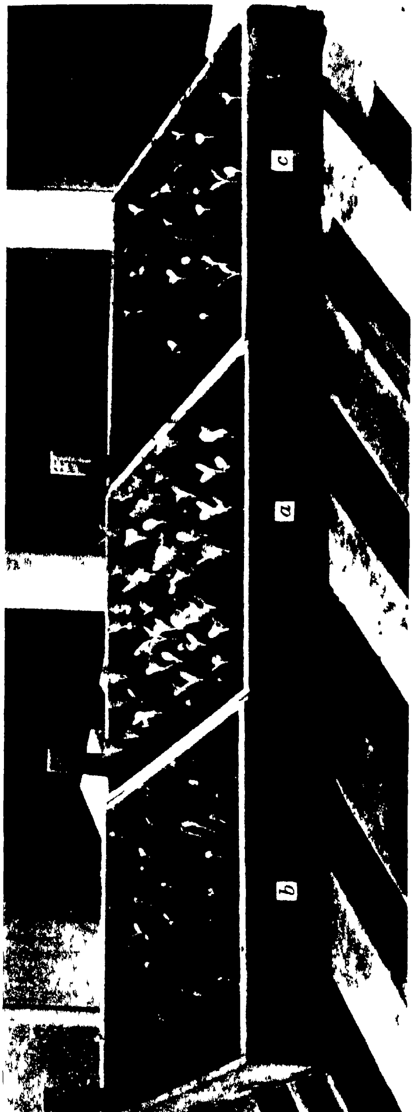

Fig. 1

WHITE.—The Sterilization of Lettuce Seed (pp. 767–780).

VEIN CLEARING AND VEIN BANDING INDUCED BY HYOSCYAMUS III DISEASE

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(With Plate XXX and 3 Text-figures)

CONTENTS

INTRODUCTION

A CLEARING or yellowing of those areas of the leaf immediately aligning the veins is a frequent sign of virus infection. With many viruses and in many hosts it is the first symptom to become visible macroscopically. A virus may produce this symptom almost invariably in some hosts but never in others. It can sometimes be encouraged or precluded by varying the conditions of growth, as for instance in curly top of sugar beet (Severin, 1929) where transparent venation occurs under glasshouse conditions. The symptom is usually short lived. In tomato infected with aucuba mosaic disease (Henderson Smith, 1928) it is soon replaced by a mottle. In tobacco infected with tobacco streak virus (Johnson, 1936) vein clearing is followed by necrosis. This paper is concerned with tobacco infected with Hyoscyamus III disease (Hamilton, 1932). In this case, vein clearing (Pl. XXX, fig. 1) may be followed later by vein banding (Pl. XXX, fig. 2). A few days after inoculation a strip up to 1 mm. in width along each side of each of the principal veins becomes yellow. The cleared condition persists for about 2 days. Then the yellow regions become diffuse and, after a further week or so, dark green bands appear along the whole or part of the length of many of the veins. Those areas

where banding occurs were thus previously devoid of colour. The bands vary in width from very narrow to several millimetres.

What are the anatomical or cytological changes which result in these macroscopic differences? Clearing might be brought about by a proliferation of the vascular strand or by inhibition or retardation of the development of the plastid primordia, of the plastids or the pigment itself. As clearing is followed by banding any destructive process would need to be followed by a process of regeneration.

Methods

For microchemical tests and certain types of examination, hand sections, frozen sections and whole mounts were used. Hand and frozen sections, unless for microchemical tests, were mounted in isotonic salt or sugar solution. For most of the study, material was fixed, embedded and sectioned by the microtome.

Young tobacco plants were inoculated with Hy. III disease. Immediately vein clearing became visible macroscopically in *one* leaf, that leaf and all the younger ones were cut up and fixed. For vein banding, leaves showing this symptom were cut into small portions each containing a vein surrounded by dark green tissue at the margin of which a small amount of the yellower tissue was left. These pieces were fixed immediately.

For general fixation, Zenker's fluid was best at all stages. For cytological details, such as plastid development, Champy's fluid gave the best results. Some material was dehydrated, cleared in cedar-wood oil, embedded in paraffin wax and sectioned in the usual way at 6–15μ. Whole mounts were made by staining the fixed material in bulk, clearing in dioxan and mounting in dioxan balsam. The stains used chiefly for the sections were crystal violet and erythrosin, safranin-light green or Feulgen's reagent with light green or orange G after Zenker fixation. After Champy's fluid, Heidenhain's haematoxylin or aniline-fuchsin-picric acid were employed. Fixed material to be mounted whole was stained in Feulgen's reagent and counter-stained with orange G, light green or picric acid.

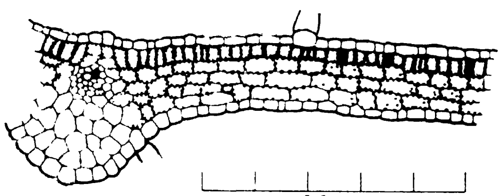
Description

As a preliminary both vein-cleared and vein-banded leaves were tested for the presence of starch. Leaves were taken from plants in the glass-houses at 4 p.m., dipped in boiling water, decolorized in alcohol and stained in iodine solution. Leaves so treated presented a striking picture.

In the younger series, a meshwork of unstained tissue contrasted with the blue colour of the interveinal areas. In the older series, dense black areas closely aligning the veins stood out against the now paler blue interveinal regions. The absence of starch from the cleared parts, its abundance in the banded parts, and its relative scantiness in the yellowish regions of the banded leaves, indicate the abnormal colouring of the leaves to be due to abnormal development or distribution of the chloroplasts and not to any masking effect.

Anatomy of the leaves

The anatomy of the leaves was examined, special attention being paid to the vascular strand. Phloem is visibly affected by many viruses. Phloem necrosis occurs in the potato (Quanjer, 1913), and "transparent venation" in sugar beet may be correlated with hypoplasia in the phloem (Esau, 1933). As in plants infected with Hy. III disease the clearing is

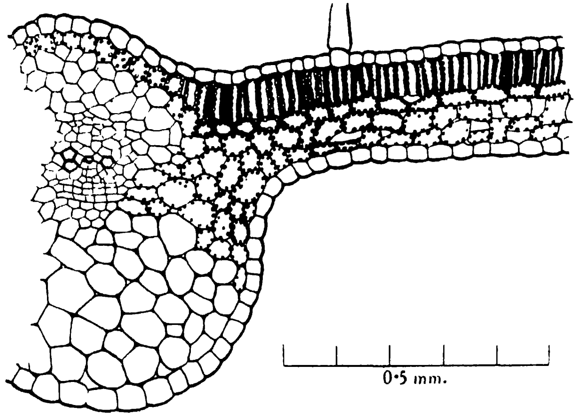


Text-fig. 1. Transverse section of infected leaf showing vein clearing.

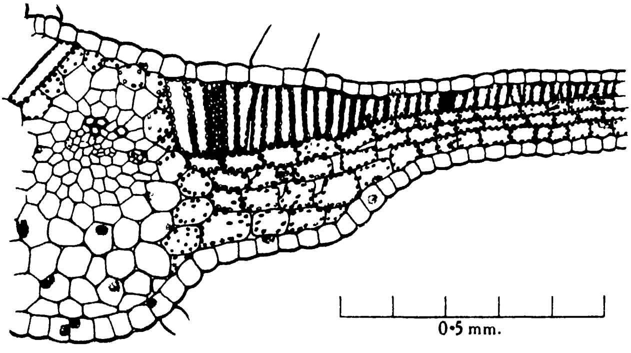
confined to those areas immediately approximating the veins, it seemed possible that some abnormality might occur in the phloem. However, an exhaustive examination of the development and structure of the vascular strand threw no light on the origin of the cleared areas. In the earlier stages abnormalities were apparent neither in the vascular strand nor in the general anatomy of the leaf (Text-fig. 1). The smaller vascular strands each consist of three or four tracheids and a few phloem cells and are completely surrounded by spherical parenchymatous cells. The assimilatory tissue is not at this stage fully developed and consists of closely packed cells usually roughly cubical or cuboidal in shape. At the time vein clearing becomes apparent these contain many plastids. The latter are present in approximately equal numbers in the cleared and in the green areas.

When vein clearing becomes apparent almost all cell division has ceased, but the cells are still growing in size. By the time vein banding is apparent, it is obvious that the leaf development has for the most part

been retarded although in certain cells growth has been excessive. Banded leaves are stunted when compared with corresponding ones of normal plants. Anatomically the vascular strand is still normal but the structure of the interveinal areas is now quite abnormal (Text-fig. 3). The banded areas are much thicker than the surrounding yellow parts



Text-fig. 2. Transverse section of healthy leaf of same age as lea in Text-fig. 3.



Text-fig. 3. Transverse section of infected leaf through a very narrowly banded vein.

of the leaf, the palisade cells in the former being more elongated than those in the normal leaf (Text-fig. 2), whilst, in the latter, very little elongation has occurred.

The hyperplasia of those palisade cells aligning the vein occurs at the expense of the spherical parenchymatous cells above the vein. These are fewer in number than in a healthy leaf. This excessive growth also causes

considerable widening of the bulge which normally occurs over the vascular bundle. Below the much-elongated palisade cells are found unusually large parenchymatous cells with well-developed air spaces. In the yellower regions, the cells of the spongy parenchyma have developed but little since clearing occurred around the veins and intercellular spaces are much smaller than in the normal leaf. The differences in colour in the different parts of the leaf are now due to differences in the size of the cells and, possibly, to the amount of chlorophyll which can be accommodated in a cell of a given size.

Cytology of the leaves

On examining the cells in detail one abnormality immediately becomes apparent, not in the cleared leaves but very conspicuously in the banded ones. Intracellular inclusions occur in abundance in almost all tissues of the older diseased leaves. They are not found in the xylem but occur in large numbers in the phloem, in the epidermis, and in all types of parenchymatous tissue. (Text-fig. 3).

The anatomical examination having thrown but little light on the problem of the distribution of the chlorophyll, on examining the individual cells, special attention was paid to the development and structure of the plastids. It has been found that the yellow mottle in certain plants infected with yellow mosaic disease of tomato (Sheffield, 1933) is produced through inhibition of the usual processes of development of the chloroplasts from the proplastids. It was thought that vein clearing might be due to some analogous cause. However, so far as can be determined visually, the plastids of plants infected with Hy. III disease develop from the primordia in the normal way. The proplastids, at first minute bodies indistinguishable from the chondriosomes, become vacuolate and increase in size until ultimately mature plastids are formed. These are lenticular in shape and consist of an almost colourless stroma in which are embedded minute coloured lenticular particles which presumably contain the pigments. Plastids from the cleared areas give the impression of being yellowish in colour rather than green, as are those from the normal areas. The minute lenticular granules can be seen dispersed throughout the plastids but they are too small for it to be possible to determine whether they are of their usual depth of colour.

Plastids are present in cleared areas and in the corresponding position in healthy leaves in approximately equal numbers. There is, however, some variation in size. Plastids vary in size in any one leaf, especially according to their position. Slight variation occurs within a single cell.

In a healthy leaf plastids from the spherical parenchymatous cells near the veins are usually about 3 μ in their largest diameter. In the palisade cells the longest diameter of the plastids may reach as much as 6–7 μ. In the cleared areas of the infected leaves the plastids over the vascular strand may be only 2 μ in diameter, and they are seldom more than 4 μ in the palisade cells. A few days later, when "banding" has replaced "clearing", the average long diameter of the plastids in these same cells is often greater than that of the plastids in the healthy leaf.

Pigmentation of the plastids

It is evident from the occurrence of so little structural abnormality to account for the yellowing of the leaf areas near the veins subsequent to infection, that the inhibiting influence comes into action at a later stage than in the case of aucuba-infected plants. It suggests that the development of the pigment may be in some way abnormal. Cleared areas were therefore dissected under the microscope from infected leaves and similar portions were taken from healthy leaves. The chlorophyllan test was carried out on both. On treatment with glacial acetic acid, clusters of straight needle-like crystals were produced by the healthy tissue. The crystals were soluble in hot glacial acetic acid, chloroform, ether, or alcohol and insoluble in potassium hydroxide. From diseased tissue similar crystals were obtained but in very small numbers. Their production by diseased cells may be due to the presence of small amounts of pigment in the diseased areas but is more likely due to the difficulty of completely isolating a "cleared" area from the attached green cells. Tests for carotin and xanthophyll were also carried out but were far from satisfactory, as very small amounts were obtained even from healthy plants. Material, dissected as before, was put into chloroform; after a few minutes either petrol ether or methyl alcohol was added. The former resulted in the separation of xanthophyll and the latter in the separation of carotin. No differences were observed in the amounts of these pigments obtained from different sources.

Distribution of the virus

The microscopic examination suggested that the action of the virus is first to delay the production of chlorophyll in those parts of the leaves which it first reaches. It then spreads from the veins across the interveinal areas. The areas near the veins are now able to develop further, whilst cell development is retarded in the interveinal regions. It seemed possible that the virus is at first present around the veins in great concentration and then, as "clearing" gives place to "banding", the virus

not only spreads from this region but is actually withdrawn from it. Experiments were designed to test this hypothesis.

Exp. I. The virus content of cleared areas of young leaves was compared with that of the banded regions of older leaves. Strips were dissected from the leaves: it was impossible to measure these accurately, but all were of approximately the same area. From the "cleared" parts it was possible to cut strips only about 0·5 mm. in width if the tissue of the vein was to be excluded. Strips were taken 1 mm. in length. To calculate the volume, the leaf was assumed to be 0·25 mm. in thickness. The volume of each piece was thus $(0{\cdot}25 \times 0{\cdot}5 \times 1 \times 10^{-3})$ c.c. or (125×10^{-6}) c.c. Actually, the thickness of the banded areas is about twice as great as that of the cleared, so the volume was also twice as great and also the resulting concentration in the extract. Such a variation was immaterial as compared with the type of difference which was expected, especially as no accurate measure was available for any of the figures. 100 pieces of each type of tissue were taken. Each series was crushed, extracted with 1·25 c.c. water, giving a dilution of approximately 1/100, and inoculated into one leaf of each of six tobacco plants. After an appropriate interval the leaves were removed from the plants, killed and starch lesions tested for. The plants were kept for systemic infections. This experiment was repeated twice with the following results:

	Total nos. of lesions	
Cleared tissue. Exp. I*a*	320	553
,, ,, ,, I*b*	233	
Banded ,, ,, I*a*	98	182
,, ,, ,, I*b*	84	

All plants showed systemic infection.

Exp. II. The virus content of the banded areas was compared with that of the yellow regions between the bands. 300 pieces 1 × 1 mm. were dissected from each type of tissue. The volume of each piece was approximately $(1 \times 1 \times 0{\cdot}25 \times 10^{-3})$ c.c. or 25×10^{-5} c.c. Each series was crushed and extracted with 3 c.c. of water giving a dilution of 1/40. Each series was inoculated into one leaf on each of fifteen different plants. The green tissue gave 587 lesions against 911 from the yellow tissue. All plants showed systemic infection.

Exp. III. Tissue from "cleared", "banded" and yellow areas was compared. 200 pieces each $(1 \times 1 \times 0{\cdot}25 \times 10^{-3})$ c.c. were cut from banded and yellow areas and 400 pieces $(1 \times 0{\cdot}5 \times 0{\cdot}25 \times 10^{-3})$ c.c. from cleared parts. Each series was crushed and extracted with 5 c.c. water to give a dilution of 1/100. The three series were inoculated into randomized half tobacco leaves. The following numbers of lesions resulted:

Cleared tissue	1644
Banded ,,	553
Yellow ,,	1224

In Exps. I and III the numbers of lesions obtained from the cleared areas was regularly about three times as great as the number obtained from the banded parts, indicating a somewhat higher concentration of virus in cleared tissue than in banded. Exps. II and III show the yellow interveinal areas of older leaves to contain more virus than the banded parts and less than the cleared areas. When these experiments were designed it was expected either that very considerable differences would

be found if the clearing were due to a direct effect of the virus on the pigment or, if the initial hypothesis were untrue, then no appreciable differences were expected. To support the hypothesis differences would need to be of a greater order of size than those shown.

The differences found are, because of their constancy, not without interest and, actually, are slightly greater than the figures indicate. As large differences were expected, it was assumed in designing the experiments that all leaves were of the same thickness. Text-figs. 1 and 3 indicate the banded parts to be about twice as thick as the cleared or yellow parts, so that a dilution of 1/100 of cleared and yellow tissues was actually compared to a concentration of 1/50 of banded tissue. From unpublished data of Dr M. A. Watson, it has been estimated[1] that at the appropriate part of the curve (i.e. where about thirty lesions occur per leaf) a drop in concentration to 1/10 results in a fall in the number of lesions to 1/5–1/10. It can thence be calculated that the virus content per unit volume of cleared tissue is probably 6–11 times as great as that of banded tissue. It is possible that the virus produced around the veins may, subsequently, be withdrawn, but it seems more probable that differences in concentration are due to variations in cell size, the concentration of the virus being a function of the number of cells in a given volume of tissue. The reduction in concentration is of the right order of size to be attributable to this cause. Some micrurgical studies have suggested that a large amount of virus is produced in the cell in a short period immediately following the time infection reaches it (Sheffield, 1938). The virus presumably first reaches those cells approximating the veins and these rapidly produce large amounts of virus. The infection passes out from these cells into the interveinal areas, each cell of which may produce virus approximating in quantity to that produced by each cell near the vein. Differences in concentration are then brought about by unequal increase in cell size. Hyperplasia occurs in those cells near the vein and, consequently, the virus concentration is there greatly reduced but, in the interveinal areas, little increase in cell size seems to occur after entry of the virus. Consequently, the concentration in these cells is not much less than in the cleared parts.

Why the virus should first inhibit and later stimulate the production of pigment in some cells and retard it in other cells, and why hypertrophy should occur in some tissues and inhibited development in others, cannot yet be explained. It might be suggested that the stimulation which is often evident in infection (Sheffield, 1931, 1936) here causes hyperplasia

[1] By Mr W. G. Cochran of the Statistical Department.

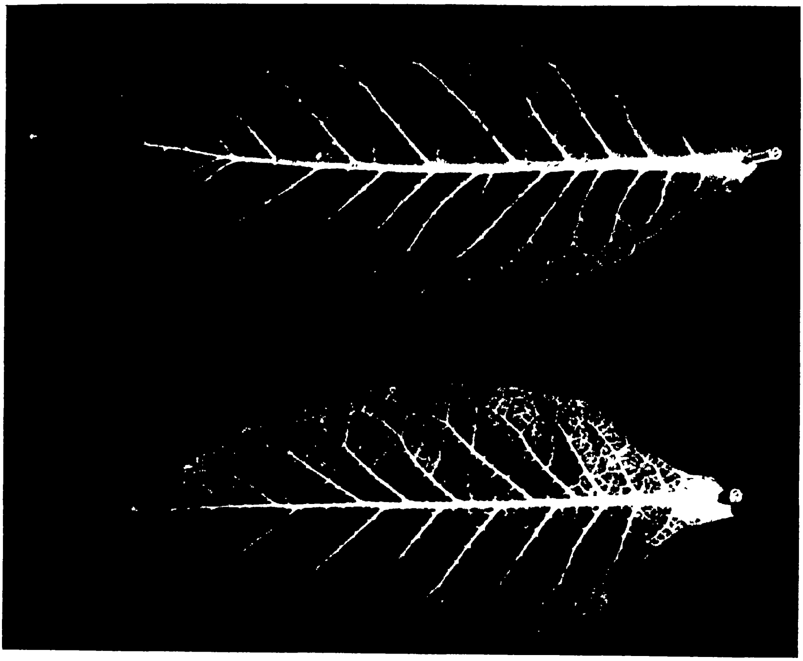


Fig. 1.

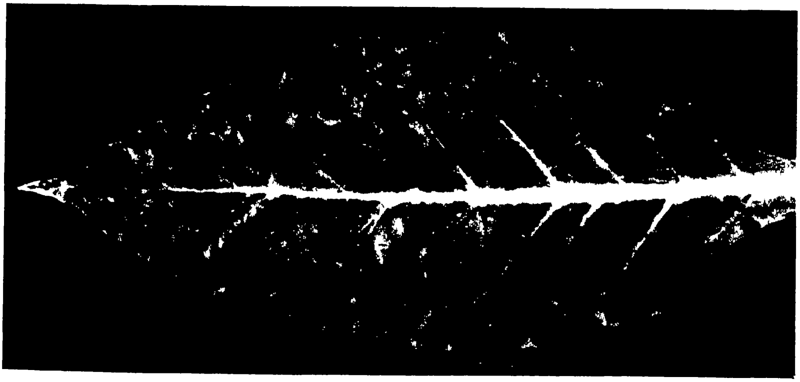


Fig. 2.

SHEFFIELD.—Vein Clearing and Vein Banding induced by Hyoscyamus III Disease (pp. 781–789).

in the cells of the banded tissue, but it is difficult to understand why the same agent should inhibit growth in other cells.

SUMMARY

The first symptom of Hy. III disease in tobacco is a clearing of the veins. This is followed later by vein banding. During clearing no anatomical or cytological abnormalities occur. The yellow colour is due to a retardation of chlorophyll formation.

When vein banding becomes apparent considerable hypertrophy is seen in the tissues near the veins and hypoplasia is apparent in the interveinal areas. Intracellular inclusions are abundant in all tissues except the xylem.

Cleared tissue contains 6–11 times as much virus per unit volume as does the banded tissue. The latter also contains less than do the yellower parts of banded leaves.

I am indebted to Fraulein Lina Cunow who made most of the permanent preparations used in this study.

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EXPLANATION OF PLATE XXX

Fig. 1. Leaf of tobacco infected with Hyoscyamus III disease, showing clearing of veins (control leaf above).

Fig. 2. Leaf of tobacco infected with Hyoscyamus III disease, showing vein banding. (Reduced by ⅓).

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STUDIES OF THE MOSAIC DISEASES OF CASSAVA

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(With Plates XXXI and XXXII and 1 Text-figure)

CONTENTS

INTRODUCTION

A MOSAIC disease of cassava (*Manihot utilissima* Pohl), first reported in East Africa under the name of "Kräuselkrankheit" by Warburg (1894) and studied by Zimmermann (1906), is now prevalent in East Africa and the adjacent islands. More recently, it has been reported from most countries of western and central tropical Africa (Dade, 1930; Deighton, 1932; Dufrénoy & Hédin, 1929; McKinney, 1929; Pascalet, 1932; Staner, 1931), from Madagascar (Bouriquet, 1932; François, 1937), and from Java (Muller, 1931). There has been growing recognition of the serious losses that the disease entails.

That this disease is caused by a virus was suggested by Zimmermann (1906), and this is now accepted by most workers, although some have sought to attribute it to a visible parasite (Kufferath & Ghesquière, 1932; Strong & Shattuck, 1930).

For general accounts of the disease in the field the papers listed under "References" may be consulted (particularly Pascalet, 1932; Staner, 1931; Bouriquet, 1932). In this paper we describe studies carried out at the East African Agricultural Research Station, Amani, mainly upon the strains of the mosaic virus and their transmission.

Since 1935 we have recognized a second disease, known as "brown streak", believed also to be caused by a virus, but one distinct from the mosaic group. A brief account of our present knowledge of this disease has been published (Storey, 1936).

SYMPTOMS AND THEIR VARIATIONS

The symptoms in cassava are characteristic of a mosaic disease—primarily chlorosis of discrete areas of the leaf-lamina. The chlorotic areas are determined during the early development of the leaf, and these areas fail to expand fully, so that stresses set up by unequal enlargement of adjacent areas cause distortion of the leaflets. The typical picture is a leaf, reduced in size, misshapen and twisted, with bright yellow areas separated by areas normally green (Pl. XXXI, fig. 1).

Great variations may be seen in the symptom manifestation, both between different plants and between different leaves of a single plant. We may analyse the symptoms as follows:

(a) *Chlorosis.* The chlorotic tissue may be a pale yellow or nearly white, with only a tinge of green; or, at the other extreme, it may be only just discernibly paler than normal.

(b) *Size of the chlorotic areas.* A "chlorotic area", although containing small "islands" of green tissue, particularly along the veins, is usually well demarcated from the green areas. It may vary in size from that of the whole leaflet (Pl. XXXII, fig. 4) to small flecks or spots (Pl. XXXII, fig. 3).

(c) *Frequency of the chlorotic areas.* All leaflets may show a nearly uniform mosaic-pattern (Pl. XXXI, figs. 1 and 2); or the mosaic-pattern may be localized in a few areas only (Pl. XXXII, fig. 4). Often chlorosis is confined to the base of the leaflet (Pl. XXXI, fig. 3); this is found, not only during early development following infection, when it would be well understood, but also in plants long systemically infected.

(d) *Distortion.*

(e) *Reduction in size of leaflets.*

(f) *General stunting.*

These last three characters all appear to be secondary, in that their extent in the leaf, or in the plant as a whole, can be related to the severity

of the symptoms under (*a*) to (*c*). Thus a severely chlorotic area grows less than one mildly chlorotic, so that the leaflet is smaller and more distorted. A plant with only mild chlorosis, or with severe chlorosis affecting only a small proportion of its total leaf area, makes stronger growth than one severely affected.

To relate the observed variations in symptoms to their causes has proved a difficult problem. One reason is the existence of more than one strain of the virus: the differences in the symptoms characteristic of the virus strains will be considered in the next section. Another reason is to be found in the different degrees of tolerance inherent in the cassava varieties. Although at present we have little exact evidence on varietal response, we can quote one striking example: the virus producing in an arborescent cassava variety only a mild localized mosaic, on transfer by grafting to the Mbarika variety, produced a typical severe mosaic.

However, within plants of a single clone, carrying a single virus strain (or conceivably mixture of strains) differences in symptoms occur from time to time or from place to place. Even in the successive leaves of a single shoot differences may often be seen as great as those of the shoot illustrated in Pl. XXXI, fig. 3, which, between stunted leaves predominantly chlorotic, bore one leaf about half chlorotic and one normal in size and colour except for a few chlorotic flecks. This kind of effect may sometimes give the appearance of complete recovery. Field experience suggests that true recovery may occur in nature, as Deighton has reported (1935); but none of the plants that we have studied has failed to revert to the diseased condition. Differences in the severity of the chlorosis may be observed, particularly with the "mild" virus strains (see next section). A single mild strain in a single clone may sometimes produce relatively severe symptoms (Pl. XXXII, fig. 2), sometimes only a faint paleing of the normal green (Pl. XXXI, fig. 2), and at yet other times no chlorosis whatever.

In part, symptom expression is determined by the age of the affected shoot. The first few leaves produced by a cutting carrying the virus are often symptomless. Thereafter the severest symptoms characteristic of the particular strain usually appear; but then as the plant grows there is often a tendency for the severity to diminish. If the shoot be cut back, severe symptoms generally reappear in the young growth from axillary buds. When newly infected a plant tends to pass through an early severe phase from which later it partly recovers.

The environment as a whole can greatly influence the symptom expression in a single clone affected by one virus strain or mixture of

strains. It is uncertain how far nutrition, water supply and illumination may have contributed to the differences that we have observed. Probably, temperature is the most important controlling factor and, in general, the lower the temperature the more severe the manifestation. There is an indication, however, that temperature may not affect all the virus strains similarly.

VIRUS STRAINS

The large influence that external factors may exert on symptom manifestation shows the need for caution in any attempt to differentiate strains of the mosaic virus on a basis of symptoms alone. We have, however, at present no other means of differentiation. The best that can be done is to study the symptoms in plants of a single clone, all grown under identical conditions.

Early in 1935 we collected in the field near Amani forty cuttings from diseased plants showing as wide a range of symptoms as could be found. After preliminary study, twenty-two of these were grafted on to plants of a healthy clone of the variety Mbarika. Duplicate cuttings of all the Mbarika plants, so infected, were planted on the same day in the greenhouse; and at intervals of a few months further cuttings were taken and planted. The symptoms in these plants were observed through 15 months. From time to time a single plant might exhibit considerable variation in its symptoms but, at any one time, the symptoms in the duplicate plants of similar age and having a single origin were closely similar.

These studies have enabled us to divide the material into two groups. Since the only known variable in the material was the causative virus, we have made a corresponding division into two groups of virus strains. The characteristic symptoms produced by each, under the special environmental conditions, are given below.

Conditions of study. Nutrition apparently optimal. In glasshouse, at lat. 5° S. and 3000 ft. altitude; overhead slatted shade obstructing 50% of sky, during hours of sunshine. Temperature uncontrolled: approximately as follows:

Annual mean: 24° C.

Hottest month: mean of daily maxima, 32° C.; mean daily range, 11·5° C.

Coldest month: mean of daily minima, 17° C.; mean daily range, 8° C.

Severe group. Chlorosis severe, yellow or sometimes nearly white; chlorotic areas usually large, usually more or less uniformly distributed,

but sometimes localized. Type G 1 (Pl. XXXI, fig. 1). Other severe types have conformed fairly closely to G 1, except that some have shown a greater tendency for the pattern to be localized to small areas of the leaf.

Mild group. Chlorosis slight, affected areas only slightly paler than normal; chlorotic areas small, either generally distributed or localized. Often symptomless. Type D 2 (Pl. XXXI, fig. 2). Other types, although included in this group, showed some differences from D 2. Thus D 11, although showing the same kind of symptoms (Pl. XXXII, fig. 1), was usually more severe than the D 2 growing alongside; but occasionally it was less severe. It was less frequently symptomless than D 2. G 21 only rarely produced faint flecks and was usually symptomless. D 7 and D 8 were always symptomless in the greenhouse, and were doubtfully placed in this group on the evidence of indefinite symptoms shown in the field. [The difference between D 2 and D 11 was more marked when these plants were grown in the open at Amani, in a valley subject to cold-air drainage; here D 2 showed a moderately severe greenish mosaic (Pl. XXXII, fig. 2); while at the same time D 11 showed only faint flecks or was symptomless.]

The difference between the symptoms produced by the severe and mild strains is so great that the validity of separating them into the two groups can hardly be questioned, even though some of the material studied may have contained a mixture of strains. So long as we lack any means of analysing a mixture, or any means of differentiation better than the study of symptoms under highly artificial conditions, no purpose would be served by an attempt at subdivision beyond the two groups. We must, however, recognize a possibility that strains, indistinguishable by the symptoms they produce, may possess varying virulence for the several cassava types. On this basis we attempted to explain the observation that certain cassavas highly resistant to mosaic in West Africa were susceptible in the Amani district (Storey, 1935); but recent evidence suggests that these same varieties may not possess as high a resistance in West Africa as was at first attributed to them (Lloyd Williams, *in litt.*).

We have encountered no certain evidence that variant strains have arisen spontaneously during the period of our study. Although the fluctuations observed from time to time in symptom expression might be interpreted in this sense, at the end of the observation period each selected strain (or mixture) produced symptoms that were on the average no different from those at the start.

TRANSMISSION

General

Bouriquet (1932), Deighton (1935), Hédin (1931) and Lefevre (1935) have reported unsuccessful attempts to transmit a cassava mosaic virus through the soil and the true seed. Without especially studying these points, we have much incidental confirmatory evidence.

All observers agree that the virus is carried in cuttings from diseased plants. Cassava mosaic is somewhat unusual among virus diseases, however, for the frequency with which exceptions to this rule are encountered. In our experience diseased plants have often grown from cuttings taken from an apparently healthy plant (cf. Pascalet, 1932); but we have no reason for attributing this to anything beyond an original failure in diagnosis, due to a too recent infection or to a suppression of symptoms. Apparently healthy plants have grown from cuttings from a diseased plant. In part, this may be attributed to the particular path followed by the virus in moving through the plant (see later). Occasionally, a cutting has produced a healthy plant, while portions of the same stem from above and below this cutting have produced diseased ones. It appears that the virus may not always be fully systemic.

The virus is transmitted across a graft (Deighton, 1932; Pascalet, 1932; Zimmermann, 1906). We have used grafting for several years as a routine technique with consistent success.

The majority of workers have failed to transmit this virus by mechanical inoculation, although successes have been reported by Hédin (1931), Kufferath & Ghesquière (1932) and Lefevre (1935). All our trials have failed, including those where the inoculation was made into immature tissue, which, as we shall show, is alone inoculable by insect vectors. In these experiments mixed juice, strained but not filtered, pressed from plants showing both severe and mild forms of mosaic, was inoculated into 10 Mbarika plants by each of the following methods:

(1) By needle scratch and puncture into the young leaflets.
(2) By rubbing the young leaflets.
(3) By hypodermic injection into the petiole of a young leaf.
(4) By hypodermic injection into the stem near the youngest leaf.

All forty inoculated plants, as well as forty controls, were still healthy two months after the inoculation.

Insect vectors

(1) *General.* Transmission of a cassava mosaic virus by a species of white fly (*Aleurodidae*) was first reported by Ghesquière (1932) in the Belgian Congo; this was confirmed at Amani (Storey, 1934) and in Nigeria by Golding (1936). Other insects are rarely found on cassava in the Amani district. We collected small numbers of three Jassids, *Erythroneura cassavae* China (1930), *Penthimia bella* Stal and *Penthimia vinula* Stal, and bred the first two successfully on cassava. In a limited series of trials, too few to be conclusive, none of these species transmitted a mosaic virus.

Our early trials of the white fly suggested peculiar features in the transmission process by this vector, in that all early experiments (not here recorded) failed if single-leaf cages were used, and success resulted only from general infestation of the plants. Subsequent studies, as detailed below, have revealed the conditions that must be satisfied for successful transmission and enabled us to evolve a reliable and convenient experimental technique.

(2) *Experiments on the conditions for infection.* All experiments were performed in gauze-protected greenhouses. Plants were of the variety Mbarika, and, in all but the earliest experiments, were the vegetative progeny of a single cutting. Control plants were raised either from alternate cuttings from the same stems, or from a random sample of the cuttings. Unless otherwise stated the insects used were wild adult white flies, collected on the same or the previous day on diseased cassava. The experimental plants were recorded as "healthy" or "diseased", without regard to whether the symptoms were severe, mild or intermediate.

The first trial by general infestation was performed by releasing several thousand white flies into a greenhouse compartment containing twenty-eight healthy Mbarika plants. Mosaic symptoms appeared on some plants within a month and after 5 months twenty-seven were diseased. Twenty-eight control plants in an adjacent compartment, not infested with white flies, all remained healthy.

When the plants were caged individually, two practical difficulties were encountered: (*a*) cassava is intolerant of the conditions in a small cage, rapidly becoming oedematous (Wolfe & Lloyd, 1912) and so abnormal that diagnosis of mosaic is difficult, and (*b*) we have found no treatment, not injurious to the plants, that kills the eggs and larval stages of the white fly. The difficulties were overcome by caging young plants (in Dietz lamp glasses) for 1 week only, whereupon the glasses were removed and surviving adult insects captured with a bush dipped

in soap solution. The plants then continued their development in the open greenhouse. Meanwhile larvae of the white fly hatched from eggs laid on the leaves, but since the larvae came to rest nearby, they could be disregarded until the pupae were nearly mature. Therefore, after a further fortnight, all the leaves bearing pupae were cut off, so that a general infestation of the greenhouse by adults was prevented. The plants, having meanwhile produced a crop of new leaves, were not considerably checked in growth by the loss of a few old ones.

Two series of experiments by this method, in which each plant received 50–100 white flies, gave six infections out of eight, and four out of five respectively. Control plants similarly caged and defoliated remained healthy.

The same procedure was now applied to the shoot-tips of older plants, the lamp-glasses being closed below by a large cork, slotted to allow the entry of the stem. One hundred white flies in each glass caused two infections in three plants. In a comparative series on similar lines, shoot-tips and single mature leaves were exposed in the cages. Five out of six plants were infected through the shoot-tip, and none out of six through a mature leaf.

In an experiment to decide which tissue of the shoot-tip could be inoculated successfully by the insects, the lamp-glasses were replaced by glass tubes, 10 cm. long by 1·5 cm. bore: otherwise the procedure was unaltered. When 100 insects were caged by this method with a young leaf less than one-quarter grown, all of five plants became diseased. When the whole shoot-tip was similarly caged, four out of five plants became diseased.

The small glass tube, placed over a young leaf, was used in all later experiments. About 100 insects were introduced into each tube.

The period of exposure of the leaf to the insects was now cut down from the 7 days previously given to 1, 2 and 4 days. The infections obtained were: 1 day, 4 out of 4; 2 days, 3 out of 4; 4 days, 4 out of 4.

In the earlier experiments the exposed leaves had been cut off after two to three weeks from the start. In order to decide whether this delay is necessary for infection, young leaves, after exposure for 2 days to the insects, were cut off after 2, 4 and 8 days from the start. No plant became diseased. The experiment was repeated at intervals of 2, 4, 8 and 16 days from the start; all of the last series and two out of three plants cut at 8 days became diseased, the first two series remaining healthy. We may thus place the threshold period for passage of the virus from the leaf into the stem at near to 8 days.

In order to determine the age at which the leaf ceases to be inoculable by the insect, we exposed a graded series of leaves, from the youngest mature one upwards. The results of this experiment appear in Table I. No infections were obtained through any leaves below the third above the youngest mature leaf. In the third column of Table I appear figures for a mean leaf ratio, representing the proportion of the leaf's full linear growth completed at the start of the experiment. The values rest on the assumption that the successive leaves have equal potentialities for growth, which in plants at the stage selected is near to the truth. In series 4, three plants out of four were infected through leaves with a mean ratio of 0·36. The individual values were: successful, 0·37, 0·37, 0·28; unsuccessful, 0·42. There is, thus, an indication that the critical stage is reached at near to 0·4. In practice however we have adopted 0·25 as a safe figure.

Table I. *Age at which cassava leaves cease to be inoculable by the insect*

Series no.	Type of leaf exposed	Mean leaf ratio and standard error*	No. of plants exposed	No. of plants infected
1	Youngest mature leaf	1	4	0
2	First above youngest mature leaf	$0{\cdot}87 \pm 0{\cdot}023$	4	0
3	Second above youngest mature leaf	$0{\cdot}70 \pm 0{\cdot}037$	4	0
4	Third above youngest mature leaf	$0{\cdot}36 \pm 0{\cdot}029$	4	3
5	Fourth above youngest mature leaf	$0{\cdot}20 \pm 0{\cdot}029$	4	4

* Ratio calculated for each plant as

$$\frac{\text{Length of middle leaflet of exposed leaf}}{\text{Length of middle leaflet of youngest mature leaf}}.$$

(3) *A successful technique.* In the course of the preceding studies we evolved a technique that is convenient, economical and reliable. We give below exact details of the procedure.

(i) Choose a leaf not more than one-quarter grown (Text-fig. 1*a*), shorten its leaflets if necessary and cut off the shoot-tip above this leaf (Text-fig. 1*b*).

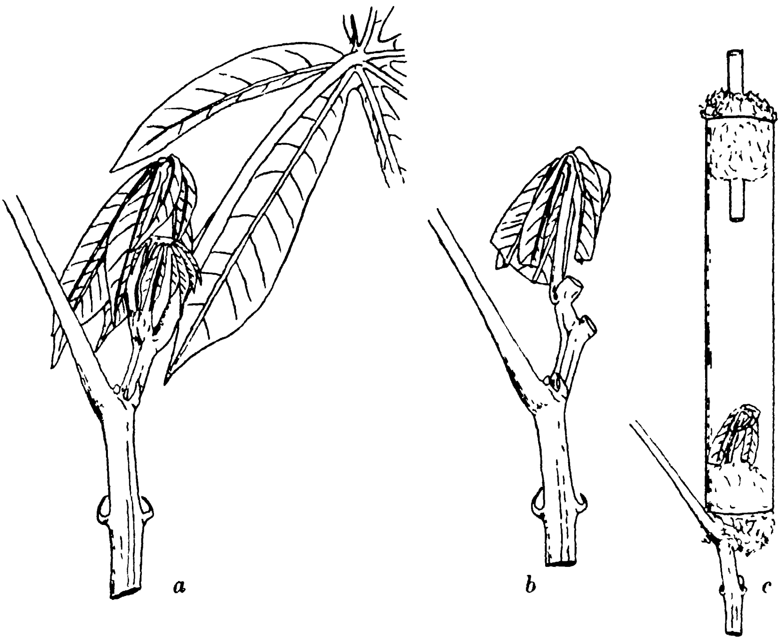
(ii) Wrap the base of the petiole with wool and place over it the glass-tube (about 10 by 1·5 cm. bore) (Text-fig. 1*c*). Secure the tube by a rubber band to an upright stick.

(iii) Blow in the insects from a pipette, connected by a rubber tube to a short length of glass-tubing passing through the upper wool plug (Text-fig. 1*c*). This glass-tubing may now be withdrawn, the hole through

which it passed closing naturally by the resilience of the wool. Shade the tube from direct sunlight.

(iv) After two days remove the plant from the greenhouse, withdraw the tube gently and capture all adult insects with a bush dipped in soap solution. The exposed leaf must not be injured during this treatment. Return the plant to the greenhouse.

(v) After a further 14 days cut off the leaf at the base of the petiole.



Text-fig. 1. Technique for experiments in transmission of cassava-mosaic virus by white flies. *a*. Shoot tip at a suitable stage for experiment. The next to the youngest leaf is about one-quarter grown. ×2. *b*. The same shoot tip, with the youngest leaf and growing point removed and one older leaf removed. Leaflets of the selected leaf shortened. ×2. *c*. Tube placed over the selected leaf, with wool plugs and small piece of glass tubing for introducing the insects. Support for the tube is not shown. ×1.

Under our greenhouse conditions, symptoms of mosaic have begun to appear in the new terminal growth after 12–20 days from the start. The method is effective with other varieties besides Mbarika. Ten plants each of Malindi and Mpezaze, varieties reputed to be more than normally resistant in the field, were exposed to 100 white flies each by this method. Nine plants of each variety were infected.

(4) *Transmission of different virus strains.* The insects used in the preceding experiments, having been collected on diseased plants in the field, were likely to be carrying both severe and mild strains of the virus. Although, once they were certainly diseased the plants were usually not further studied, the majority showed severe symptoms, and there could be no doubt of the insects' ability to transmit a severe strain of the virus. A few plants, however, developed mild symptoms suggesting that the insects had here transmitted only a mild strain. Their ability to do so was confirmed in the following experiments.

A mixed non-infective culture of white flies was established by repeated transfers to healthy plants of a *Phaseolus* sp. A test of a sample of these insects gave no infections in six cassava plants[1]. Insects from this culture were transferred to an Mbarika plant carrying the D 2 mild virus strain, and allowed to develop for 6 months. A test of this second culture gave six infections in six Mbarika plants, all showing the typical symptoms of the D 2 strain.

(5) *Identity of the vectors.* Ghesquière (1932) referred the vector in the Belgian Congo to his *Bemisia mosaicivecta* sp.n. (a misprint, as he informs us by letter, for "*mosaicivectura*"). Later (1934), Mayné and he listed this insect under the designation *B. gossypiperda* Misra and Lamba var. *mosaicivectura*. Specimens from a field collection at Amani submitted to M. Ghesquière were determined as identical with his.

Three inbred cultures of the insects were raised on *Phaseolus* spp. from single wild females. Their ability to transmit was tested in three greenhouse compartments, containing surface-sterilized cuttings of Mbarika planted in steam-sterilized soil. Each insect culture was established on diseased plants in each compartment, and left to migrate naturally to the healthy plants. The results were: Culture A caused infection of seven out of ten plants; culture B, two out of ten; and culture C, none out of ten. Control plants in a compartment adjacent to A remained healthy and free from white flies.

Specimens of culture A were submitted to Mr Karam Singh Lamba (one of the authors of *Bemisia gossypiperda* (Misra & Lamba, 1929)), and through the Director of the Imperial Institute of Entomology, to Mr G. H. Corbett. Mr Karam Singh reported that our material appeared

[1] Very few of the insects survived the two days of this test. This has been our usual experience when this white fly is transferred to a new species of food-plant. The control test is therefore not conclusive, but does not detract from the evidence that the white fly can transmit a mild virus strain.

to him to be *B. gossypiperda.* Mr Corbett identified it as *Bemisia* sp. near *nigeriensis* Corb.

Golding (1936) refers the insects of his trials to *B. nigeriensis* Corb.

In view of the difficulties that this group of insects presents to the systematists, it remains in doubt whether one or several species of *Bemisia* transmit the virus of cassava mosaic; and, indeed, whether the same or different species transmit certain other African viruses affecting tobacco (Storey, 1931) and cotton (Kirkpatrick, 1930).

DISSEMINATION OF THE VIRUS THROUGH THE PLANT

We have studied the spread of the virus through a recently infected plant by growing, as cuttings, sections of its stem. Twenty-eight plants, selected in an experimental plot as having developed during the preceding month the first symptoms of mosaic, were thus found to have virus right to the base of the diseased shoots, even though some were as long as 8½ ft. Fifteen of the plants bore also apparently healthy shoots arising from the same original cuttings; six of these shoots were free from virus, seven contained virus throughout their length, and two gave diseased plants from their lower cuttings and healthy ones from the upper cuttings. Although the virus travels down to the stem rapidly, its movement across the original cutting and into other shoots may be relatively slow.

Similarly, the movement into side branches borne on the infected shoot appears to be slow. One or two apparently healthy branches borne by eleven otherwise diseased shoots were tested, and all were found to be virus-free except those from four shoots. One of these gave diseased plants from the lower cuttings and healthy from the upper, and three gave all diseased plants. Since all branches of the original plants were exposed to an equal chance of natural infection, some of the last may have been infected independently before cutting, although too recently to have developed symptoms. There is, evidently, a marked tendency for the virus to move down a shoot, passing on its way the origin of a branch and yet failing to move up into it. On one plant the virus-free branch arose within 2½ ft. of the top of a shoot 8½ ft. long, which contained virus right to its base.

INTERACTION OF VIRUS STRAINS

Recent work (Kunkel, 1934; Salaman, 1933; Thung, 1931) has shown that a mild strain of a virus can sometimes confer immunity from a severe strain. The mild strains of the cassava mosaic virus here studied,

however, gave no evidence of conferring immunity when a severe strain was later introduced by grafting. A large number of experiments were performed, in which stocks of the variety Mbarika, previously infected by grafting with the mild strains D 2, D 7, D 8 and D 11, received scions carrying a severe strain. In every experiment symptoms of the severe strain appeared in the new growth from the stock. We may note, however, that Köhler (1935) has reported the breakdown of an otherwise effective immunity, when the manner of infection was through a graft.

When the severe strain was introduced by the insect vector, some indication of an interaction was obtained. In our first experiment, five Mbarika plants carrying the mild D 2 strain were exposed by the usual technique to infection by wild white flies. Five healthy Mbarika plants were similarly treated. These control plants all became diseased, and all but one showed typical severe symptoms. Of the five D 2 plants, two never showed more than the faint symptoms of the D 2 virus; one developed the severe disease and was indistinguishable from the controls; while two plants produced leaves with only small areas of severe chlorosis scattered over their surface, on which the mild symptoms of D 2 were evident (similar to Pl. XXXII, fig. 3). The one exceptional control plant was also of this type. All these plants were retained under observation for 9 months, during which time the general type of symptoms in each underwent no marked change.

When this experiment was repeated, none of 7 plants originally carrying the D 2 virus escaped infection by a severe strain. Two developed a severe disease and 5 severe flecks on a mild background (Pl. XXXII, fig. 3). The originally healthy control plants, however, showed closely similar symptoms, only one having the typical severe disease.

An evident defect in our experimental procedure was the use of wild insects, liable to be carrying both severe and mild virus strains. It is possible that the anomalous results obtained, particularly in the last experiment, may have been due to a simultaneous infection by two strains. The experiments have, however, demonstrated that no reliance can be placed on the mild strains at present available as a practical means of protection from severe strains. Field trials with cassava carrying the D 2, D 7, D 8 and D 11 strains confirmed this conclusion. Although these trials permitted no exact comparison of resistance between healthy and mildly diseased clones, they showed that all the mildly diseased ones could contract naturally the severe disease.

DISCUSSION

We have shown that mosaic disease of cassava is not due to a single uniform causative agent. It is hardly important whether, at this stage, we regard the agents as different viruses or strains of one virus. We have chosen the second course as conforming best with the modern outlook on the virus problem. The proof that one species of insect transmits viruses causing both the severe and the mild forms of the disease favours this choice. The fact that a mild strain had failed to confer immunity from a severe one does not point necessarily to a contrary choice. We have shown that there is a marked tendency for the symptoms of cassava mosaic to be confined to limited areas of the leaf surface. This may denote a similar localization of the virus. In the modern view (Salaman, 1936), protection by one strain of virus from another depends on "occupation" of the tissues; plant cells that are occupied by one strain are unavailable to another. Thus, we may suggest that in cassava a mild strain of virus may occupy only defined areas of the leaf, leaving other areas open to invasion by a severe strain. We have observed that, in a mixed infection, the symptoms of the severe disease tend to be more circumscribed than in a single infection by a severe strain alone.

Although we have reason for dividing the strains of the cassava mosaic virus into two groups and may suspect differences between members in each group, we have only touched the fringe of this problem. At present we have only a hint of the existence of strains indistinguishable by symptom examination but differing in their ability to infect the several cassava types. And yet this is the important practical problem, for it may well decide whether the extensive work of breeding for resistance now in progress will have any permanent and widespread success. But this same breeding programme may provide us with the differential hosts that are the essential tools of a sound study of virus strains.

Our study of insect transmission has revealed a situation that, for its dependence on certain rigid conditions, is probably unique. We can offer no explanation why an insect, that can successfully maintain itself on mature leaves, can inoculate successfully only immature ones. We can perhaps perceive a reason why, after inoculation, these leaves need several days to develop before the virus will pass out of them into the stem. There is a growing tendency to relate the movement of certain viruses within the plant to the translocation of carbohydrates (cf. Bennett, 1937). We may suppose that the young leaf, at the inoculable stage, is

receiving carbohydrates from other parts of the plant, and that only when its photosynthetic mechanism becomes active and the flow of carbohydrates is reversed, can the virus travel from the leaf into the stem. On similar grounds we may, perhaps, explain the rapid movement of the virus down the stem and its slow upward movement into branches.

Our insect-transmission experiments, largely successful once technical difficulties were overcome, have all been by mass infection with large groups of insects. We have no reason for attributing any particular merit to the group except that of increasing the probability of infection. We have, however, at present no conclusive evidence that a single insect is capable of inoculating a plant successfully.

SUMMARY

The symptoms of mosaic disease in cassava, although generally typical of the mosaic group, show wide variations, due in part to the varietal reaction of the plant, to its stage of development and to the environment. The most important cause of variations however is differences in the strains of the virus, of which we have recognized, through the study of symptoms, two groups of severe and mild strains.

The viruses are transmitted across a graft, but we have failed to obtain mechanical transmission by needle or hypodermic injection.

A *Bemisia* sp. can transmit both groups of strains. It can inoculate the plant only through immature leaves, less than about one-quarter of their full length. The virus so inoculated does not pass out of the leaf until about 8 days have elapsed. On the basis of this knowledge a convenient and reliable single-leaf cage technique has been developed.

After the virus has entered the stem it passes rapidly to the base of this stem, but only slowly into side branches from it or into other stems arising from the same original cutting.

Infection of a plant with a mild strain of virus failed entirely to confer immunity from infection by severe strains introduced by grafting. If the severe strains were inoculated by insects there was an indication of some conferred resistance but insufficient to make the procedure practically useful in control.

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EXPLANATION OF PLATES XXXI AND XXXII

PLATE XXXI

Fig. 1. A severe strain of the mosaic virus (no. G 1). Typical manifestation in var. Mbarika in the greenhouse. $\times \frac{5}{8}$.

Fig. 2. A mild strain (no. D2). Typical manifestation in var. Mbarika in the greenhouse. $\times \frac{7}{12}$.

[N.B. Figs. 1 and 2 are from strictly comparable leaves. That in fig. 2 is about the normal size for a healthy leaf of this variety; the reduction in size of the leaves in fig. 1 is a typical effect of the virus strain.]

Fig. 3. The shoot-tip of a diseased plant, bearing in succession (*a*) severely diseased leaves, (*b*) one leaf with severe chlorosis confined to the base of each leaflet, (*c*) a leaf entirely healthy except for a few chlorotic flecks, (*d*) a further group of severely diseased leaves. $\times \frac{1}{3}$.

PLATE XXXII

Fig. 1. A mild strain (no. D11). An example of the most severe manifestation given by this strain in var. Mbarika in the greenhouse. Under similar conditions the strain D2 rarely gives symptoms as severe as this. $\times \frac{1}{3}$.

Fig. 2. A mild strain (no. D2). The manifestation shown by var. Mbarika grown in the cool season in a valley at Amani subject to cold-air drainage. Cuttings from this plant showed the typical D2 symptoms (as fig. 2 Pl. XXXI) when grown in the greenhouse. Plants with the D11 strain growing alongside in the valley were at this time almost symptomless. $\times \frac{1}{3}$.

Fig. 3. A mild strain (no. D2) combined with a severe strain. A leaf from a plant, originally infected with the D2 strain, later exposed to infection by wild White Flies. The severe symptoms are confined to small areas of severe chlorosis, between which the mild symptoms of D2 can be faintly seen. $\times \frac{1}{3}$.

Fig. 4. A severe strain showing an extreme localization of effect to one leaflet and a part of one other. The remaining leaflets appear to be entirely healthy. $\times \frac{1}{3}$.

(*Received* 9 *May* 1938)

Fig. 1

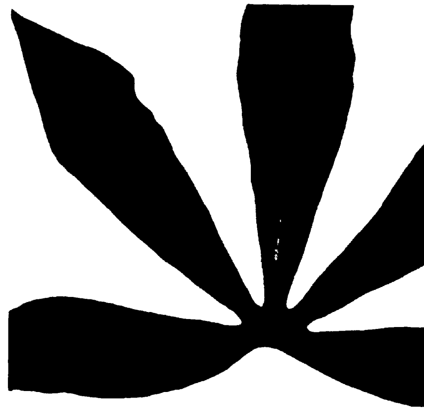


Fig. 2

Fig. 3

STOREY AND NICHOLS Mosaic Diseases of Cassava (pp 790 806)

PLATE XXXII

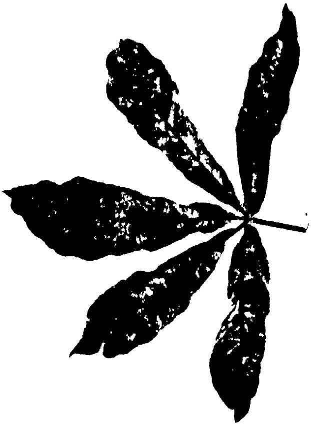


Fig. 2

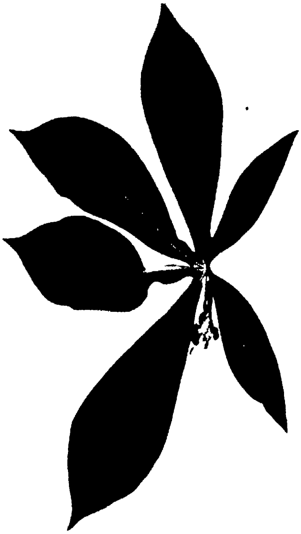
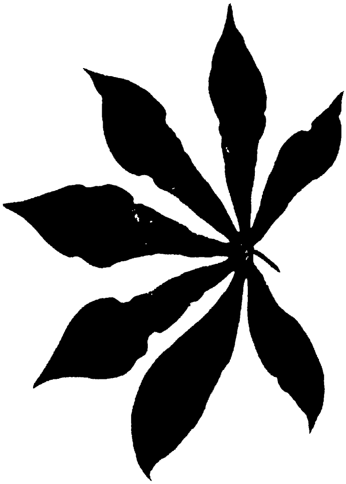


Fig. 1



STOREY AND NICHOLS. MOSAIC DISEASES OF CASSAVA (pp. 790–806)

STUDIES ON AMERICAN FOUL BROOD OF BEES

III. THE RESISTANCE OF INDIVIDUAL LARVAE TO INOCULATION WITH THE ENDOSPORES OF *BACILLUS LARVAE*

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In previous experiments it was found that colonies of bees contract American foul brood rapidly when inoculated with the spores of *Bacillus larvae*, while the vegetative cells fail to produce the disease (Tarr, 1937*a*). In these experiments the larvae were not inoculated individually, the spores or vegetative cells of *B. larvae* being either fed to the bees in syrup or sprayed over the combs of developing larvae. Such methods of inoculation are unsatisfactory for the reasons that, although direct inoculation of larvae may take place by the spraying method, both techniques present the possibility for indirect inoculation of the brood, as the causal organism is carried by adult bees; and because neither of them allow for an estimation of the minimum lethal dose of spores for a single larva. Experiments designed to ascertain the minimum lethal dose of spores by oral inoculation of individual larvae yielded the results recorded below.

Experimental

Suspensions of washed spores of *B. larvae* were prepared from ropy larvae dead of American foul brood as previously described (Tarr, 1938*b*). Two to five comb nuclei of hybrid bees prepared as in former experiments were used (Tarr, 1936). The method of inoculation of individual eggs or larvae was essentially an oral one. Combs containing eggs or brood of the age desired were removed from the colonies. From 0·001 to 0·005 ml. of spore suspension was introduced into the larval cell by means of an Agla micrometer syringe, being either placed at the base of the cell near the eggs, in the brood food surrounding the younger larvae, or near the mouth parts of the older coiled larvae. In a few instances spores were fed to recently sealed larvae by introducing the needle of the hypodermic syringe through the cell cap. The rows of cells containing the inoculated larvae were marked by driving large pins through the midrib of the combs

containing them, and the combs were returned to the bee colony immediately. It was found that, with careful inoculation, adult bees rarely remove young larvae which are fed approximately 0·001 ml. of suspension, or older larvae which are fed up to 0·005 ml. With larger volumes of inocula there is great danger that the bees will remove numbers of the inoculated larvae. In certain of the experiments the spores were sprayed over the developing brood by the technique already described (1937 *a*). Periodical examinations of the inoculated larvae were made, and, in many instances, a certain number of pupae were selected at random from the inoculated areas and examined microscopically. The method of recording inoculations and results was similar to that employed in previous work (Tarr, 1936, 1937 *a*). A large number of larvae in many different colonies were inoculated but only a limited number of typical experiments are recorded here. No attempt has been made to calculate the standard error of the spore number in the case of each inoculum for the reason that, as has already been shown (Tarr, 1938 *b*), it is unlikely to exceed $\pm 5\%$ with spore suspensions containing over 1000 spores per ml. with good counting and dilution methods.

Exp. 1. Date of inoculation 25 June.

Each of twenty-five eggs and young larvae about 1 day old, in four separate groups in adjacent cells, were fed 0·005 ml. of spore suspensions of *B. larvae* so that the individual larvae in the various groups received approximately 44×10^2, 44×10^3, 44×10^4 and 44×10^5 spores respectively.

Results. 6 *days*: A number of inoculated larvae had been removed by the bees (probably about 25%), the remainder had been sealed over and no infected larvae were seen. 8 *days*: No diseased larvae were seen. Three sealed pupae from each group were selected at random and were examined microscopically: no spores or vegetative cells of *B. larvae* were detected in them. Subsequent examinations 18, 22, 27 and 32 days after inoculation revealed no infected larvae, the colony remaining free from American foul brood.

Exp. 2. Date of inoculation 28 June.

Individual larvae about 1 day old and eggs in three groups of sixty were fed 0·005 ml. portions of spores suspensions so that each received approximately 10×10^4, 10×10^5 and 10×10^6 spores respectively.

Results. 3 *days*: Very few larvae had been removed by the bees, and most were ready for sealing; no diseased larvae were seen. Further examinations of the brood 9, 21, 37 and 65 days after inoculation revealed no infected larvae.

Exp. 3. Date of inoculation 28 June.

Individual larvae 4–5 days old in three groups of forty were inoculated with doses of spores of the same magnitude as those given in the previous experiment.

Results. 1 *day*: The inoculated larvae were being sealed by the bees and practically none had been removed. Two pupae selected at random from each group were examined

microscopically. A few spores were seen in those which had received the larger inocula, but there was no sign of germination of these, and no vegetative cells of *B. larvae* were detected. 3 *days*: Few if any of the inoculated larvae had been removed by the bees. Three pupae from each inoculated group were selected at random and examined microscopically. All appeared to be free of spores of *B. larvae*; no bacteria being seen in any of the nine preparations examined. No larvae affected with American foul brood were seen on examinations 9 and 18 days after inoculation. The colony swarmed 19 days after inoculation and was permitted to requeen itself. The brood produced by the new queen was examined 65 days after inoculation and proved to be healthy.

Exp. 4. Date of inoculation 8 July.

Eggs and young larvae about 1 day old in three adjacent groups of forty were fed 0·001 ml. of three different dilutions of a spore suspension of *B. larvae*, so that individual larvae received approximately $9{\cdot}4 \times 10^4$, $9{\cdot}4 \times 10^5$ and $9{\cdot}4 \times 10^6$ spores respectively.

Results. 3 *days*: Very few inoculated larvae had been removed by the bees and many were practically ready for sealing. Subsequent examinations 5, 11, 20, 26 and 53 days after inoculation revealed no larvae affected with American foul brood.

Exp. 5. Date of inoculation 20 July.

One-hundred, 4–5 day old unsealed larvae, and a few recently sealed larvae, were fed 0·005 ml. containing approximately 47×10^6 spores each.

Results. The larvae in the inoculated area had all been capped by the bees and few if any had been removed. Ten sealed pupae were selected at random from the inoculated portion of the comb. On microscopical examination all appeared to be free of spores or vegetative cells of *B. larvae*. 8 *days*: No infected larvae seen. 12 *days*: A few of the bees in the inoculated area emerging and apparently healthy; no diseased larvae seen. No sign of American foul brood was observed on inspection of the brood 20, 41 and 50 days after inoculation.

The results of the above experiments indicate that individual larvae possess a very high degree of resistance to simple oral inoculation with the spores of *B. larvae*. It was thought that some essential substance might have been removed on washing the spores and therefore direct inoculation with whole crushed infected larvae and also with unwashed spore suspensions were tried.

Exp. 6. Date of inoculation 19 July.

Fifty individual larvae about 4–5 days old were each fed approximately $7{\cdot}8 \times 10^5$ spores of *B. larvae* in 0·005 ml. of an unwashed suspension.

Results. 4 *days*: Few if any of the inoculated larvae had been removed by the bees and no diseased larvae were seen. Six sealed pupae from the inoculated area of the comb were selected at random, and microscopical examinations of these showed no bacteria. No sign of American foul brood was found when the colony was examined 8, 13, 21, 38 and 51 days after inoculation.

Exp. 7. Date of inoculation 28 July.

Four larvae very recently affected with American foul brood which, on microscopical examination, were found to contain practically nothing except non-sporulating vegetative cells of *B. larvae* in apparently pure culture, were ground up in 5 ml. of

distilled water. Approximately 0·005 ml. of the resulting suspension were fed to each of fifty, 4–5 day old larvae.

Results. 3 *days*: A few of the inoculated larvae had been removed by the bees and the remaining sealed brood showed no infected larvae. Subsequent examinations 6, 12, 29, 35 and 41 days after inoculation revealed no infected larvae.

In view of the failure which had accompanied all attempts to inoculate individual larvae by direct oral inoculation with relatively massive doses of *B. larvae* spores, experiments were made in order to verify previous work in which it was shown that spraying dilute spore suspensions over developing brood causes rapid spread of American foul brood (Tarr, 1937*a*). The results obtained in a typical experiment are given below.

Exp. 8. Date of inoculation 15 July.

One comb containing chiefly eggs and young larvae was removed from a three comb nucleus and the brood was sprayed with 20 ml. of an aqueous suspension containing approximately 94×10^6 spores of *B. larvae.*

Results. 7 *days*: The inoculated comb contained a number of rather dull white, flabby, chewed pupae and two slimy brown pupae. Ten of these were examined microscopically and all contained numerous vegetative cells (some sporulating) or spores of *B. larvae*, and from four this organism was isolated in pure culture. Further examinations 11 and 19 days after inoculation showed that American foul brood was spreading rapidly, and the colony was destroyed.

The results of these experiments strongly suggested that the adult bee was playing some part in transferring the disease to the larvae, and some experiments were made in an attempt to ascertain whether *B. larvae* increased in virulence during passage through the adult bee.

Exp. 9. Date of inoculation 5 August.

A number of bees which had sucked up the ropy decaying remains of larvae dead of American foul brood when streaked over the surface of a brood comb were caged for 24 hr. at 33° C. Their recta were full of spores of *B. larvae.* Four of the bees thus treated were ground up as finely as possible in 5 ml. of *M*/5 phosphate buffer *p*H 7·2. Fifty 4–5 day old larvae were each fed approximately 0·005 ml. of this suspension (the coarse debris was allowed to settle for a few minutes before the hypodermic syringe with which the larvae were inoculated was filled).

Results. 4 *days*: No diseased larvae were noticed in the inoculated area of brood comb. Nine sealed pupae from this area were examined microscopically. All but two of these were apparently free from bacteria. The remaining two showed a few rod-shaped bacteria but it is not known whether these were *B. larvae* organisms. All subsequent examinations carried out at intervals of 11, 20, 27 and 34 days after inoculation showed that the brood of the colony remained absolutely free from American foul brood.

Exp. 10. Date of inoculation 26 August.

Twenty-five young bees were taken from a healthy colony and were fed as much of a mixture containing equal portions of honey and ropy material from larvae dead of American foul brood as they would take in 24 hr. at 33° C. Eight of the bees thus treated were ground up finely in 5 ml. of sterile distilled water. The coarse debris in the resulting suspension was removed by filtering it through sterile cheese cloth. Twenty-

five 4–5 day old larvae and twenty-five 1–2 day old larvae were fed 0·005 and 0·001 ml. portions of this suspension respectively.

Results. 4 *days*: Nearly all the inoculated larvae had been sealed by the bees, very few having been removed. After 6 and 13 days the brood was again examined but no infected larvae were seen.

Similar experiments made with either ground up recta or whole digestive tracts of adult bees taken from colonies badly affected with American foul brood yielded equally negative results.

DISCUSSION

The direct inoculation of individual larvae has never proved a satisfactory method of introducing American foul brood to a colony of bees. On the other hand, European foul brood is initiated readily by this method (Tarr, 1938*a*). White (1920) stated that, "Direct inoculation (of larvae) with a capillary pipette is less satisfactory in experiments on American foul brood than in those on Sacbrood", but did not describe the experiments, the results of which led him to make this statement, in detail. Toumanoff (1929) was unable to infect individual larvae by feeding them directly with vegetative cells obtained from pure cultures of *B. larvae*. In his experiments he records that over one half the inoculated larvae were removed by the bees while the remainder matured normally. Sturtevant (1932) attempted to inoculate individual larvae, at ages ranging from about 4 days up to the time just subsequent to the sealing of their cells, by feeding them 0·01 ml. portions of sugar syrup containing *B. larvae* spores. In his experiments he returned the combs containing the inoculated larvae to the bee colonies in such a way as to keep them from contact with the adult bees for some time, thus introducing a short period of "starvation" of the larvae. With the method of inoculation used, he found that the bees invariably removed larvae which were fed approximately 25×10^6 or 50×10^6 spores, while those receiving smaller numbers were removed less frequently, depending on the length of time the larvae were kept away from the adult bees and the size of inoculum of spores. In only one of a series of nuclei used was American foul brood initiated by this method of inoculation and, in this case, the individual larvae had been fed approximately 10×10^6 spores. It seems highly likely that in this case the susceptibility of the individual larvae to infection with *B. larvae* spores was enhanced by the starvation period, for the writer showed that it is fairly easy to cause an infection in healthy larvae by feeding them large doses of certain of the secondary invading bacteria occurring in European foul brood if the larvae fed are subsequently starved at hive temperature (Tarr, 1936). When larvae thus

inoculated were returned to a healthy bee colony no disease resulted. From his experiments Sturtevant concluded that it is by no means easy to infect individual larvae with American foul brood, but did not suggest that the adult bee might play a very important part in disseminating the disease within the bee colony.

Jaeckel (1930) investigated the histological changes which occur in larvae affected with American foul brood. Only a few of the larvae which he studied were naturally infected, the majority being inoculated by placing 0·1–0·15 ml. of a suspension made from ropy larvae dead of American foul brood mixed with one or two parts of water in their cells. This method of inoculation would seem to be very crude since the average worker cell holds approximately 0·25 ml. of water, and the volume of inoculum he employed must have covered and probably chilled the larvae. Indeed, he remarks that in some experiments all the inoculated larvae were removed by the bees and, at the best, only about 20% were not removed. It is surprising that such a drastic method of inoculation ever succeeded, for the writer, in somewhat extensive experiments, has found that there is a strong tendency for the bees to remove larvae, especially the younger ones, when volumes of inocula much in excess of 0·01 ml. are employed. Jaeckel stated that he kept the combs containing inoculated larvae for a few hours at 34–37° C., until the larvae had consumed the infective material, before introducing them to bee colonies. It is practically certain that unsealed larvae would never consume a fraction of the large dose of suspension which was fed to them. From the results of his histological work Haeckel was led to state that American foul brood is inclined to be a type of septicaemia, the causal organism penetrating the epithelial cells of the stomach of the larvae and being carried by the blood to various organs. If this be so, then this disease must differ radically from European foul brood which is apparently a purely intestinal infection, the causal organism being localized in the larval gut (Tarr, 1938*a*). It is of interest that Jaeckel realized that such large inocula as he employed were extremely unlikely to occur under natural conditions, and he suggested that *B. larvae* might gain access to the larva by some route other than the oral one. Thus, he mentioned that the spiracles of the larva might offer a convenient portal of entry to the causal organism.

When the results of the work discussed above are considered in conjunction with those obtained in the present experiments it is evident that some factor exists by which the pathogenicity of *B. larvae* for its host is determined. Since indirect inoculation, by allowing the adult bees

of the colony access to dilute spore suspensions of *B. larvae*, is uniformly successful in producing American foul brood, and direct inoculation of individual larvae at all stages of growth with concentrated spore suspensions fails to cause the disease, it can only be inferred that the adult bee plays an important part in inoculating the brood of the bee colony. This role might be a purely physical one, the bee introducing spores of *B. larvae* into the larva in some manner which is not attained by simple oral inoculation; or a more complex one, the pathogenicity of the spores being changed by the adult bee. Careful histological work, such as has been carried out on larvae affected with European foul brood (Tarr, 1938*a*), might yield much valuable information regarding the manner in which *B. larvae* first invades the tissues of its host. The possibility that *B. larvae* undergoes some change, during its carriage by the adult bee, which causes it to become more virulent must be considered. So far experiments designed to test this point, which have been recorded in this paper, have yielded negative results. However, further experiments should be carried out in order to determine the fate of *B. larvae* spores in adult bees of various ages, particularly in those bees which have assumed the nursing duties of the colony. The significant part played by insect vectors in the transmission of virus diseases of plants has long been recognized. Thus streak disease of maize, which cannot be transmitted from plant to plant by simple mechanical means, is readily carried by certain species of leaf hoppers inoculated with the causal virus (Storey, 1928, 1933). It will be interesting if the adult bee proves to be an "insect vector" to its own brood.

SUMMARY

American foul brood is not produced by direct inoculation of eggs, or larvae from the time of hatching to that just subsequent to sealing, by placing from 0·001 to 0·005 ml. of aqueous suspensions of washed spores of *Bacillus larvae*, obtained from natural sources, in their cells.

With this method of oral inoculation, larvae are rarely removed by the bees and mature normally when each receive from a few thousand to over 45×10^6 spores of *B. larvae*.

Feeding individual larvae with unwashed spores of *B. larvae*, or with portions of crushed larvae recently affected with American foul brood, does not cause the disease.

Methods of inoculation, in which the adult bees are allowed access to relatively dilute suspensions of *B. larvae* spores, form a satisfactory means of initiating American foul brood in colonies of bees.

It is inferred that adult bees play an important part in carrying American foul brood in the colony, and the possible role of the adult bee as "insect vector" of American foul brood is considered.

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STUDIES ON EUROPEAN FOUL BROOD OF BEES

IV. ON THE ATTEMPTED CULTIVATION OF *BACILLUS PLUTON*, THE SUSCEPTIBILITY OF INDIVIDUAL LARVAE TO INOCULATION WITH THIS ORGANISM AND ITS LOCALIZATION WITHIN ITS HOST

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(With Plates XXXIII–XXXV)

Previous experiments verified the fact that European foul brood is a single disease caused by *Bacillus pluton* White, an organism which refused to multiply on any of a variety of nutrient substrates tested (Tarr, 1936). It was also found that the gross external symptoms of the disease could be greatly modified by introducing large numbers of certain of the bacteria which commonly occur as "secondary invaders" in this disease into recently affected colonies. It seemed important to ascertain whether *B. pluton* would grow upon the tissues of the developing chicken embryo, the minimum lethal dose of this organism for individual larvae, and by what method it invades the tissues of its host. These points have been investigated experimentally and the results obtained are recorded below.

Experimental

(i) *Attempts to cultivate* B. pluton *on the tissues of the developing chicken embryo*

The chorioallantoin membrane and embryo brei media were prepared as in experiments concerned with the growth of *B. larvae* (Tarr, 1938*a*). Suspensions of *B. pluton* were made from the stomachs of larvae recently affected with European foul brood by the technique previously described (Tarr, 1937), Tyrode or Ringer solution being used as suspending medium in most cases instead of phosphate buffer. *B. pluton* tends to form clumps, and although the larger clumps can frequently be removed by passing the suspension through a very thin layer of sterile cotton wool, it is practically impossible to obtain uniform suspensions of this organism as with *B. larvae* spores (Tarr, 1938*a*). The addition of a trace of sodium hydroxide solution together with some methylene blue facilitates the direct counts of

the organisms in such suspensions by tending to break up the clumps and to stain the cells, but such treatment cannot be used for suspensions employed for inoculation. Consequently, it has only been possible to give approximate figures for the numbers of organisms used for a given inoculation, and the figures recorded in the following tables must only be considered as such. All cultures were incubated at approximately 35° C.

Exp. 1. Four tubes containing 5 ml. of embryo brei medium were inoculated with *B. pluton* organisms, two tubes receiving approximately 40×10^6 organisms in 1 ml. of suspension, and the other two 40×10^5 in 0·1 ml. The inoculated media were incubated, microscopical examinations being made at intervals. *B. pluton* organisms could readily be demonstrated in the freshly inoculated medium, but these rapidly disappeared, perhaps owing to the lytic action of the medium or to an actual autolysis. After 3 weeks examination revealed no bacterial cells, and transfer of portions of the inoculated brei to beef digest brood filtrate agar resulted in no bacterial growth.

Exp. 2. The stomachs of four apparently healthy larvae about 3 days old which contained *B. pluton* in apparently pure culture were obtained, each gut being suspended in approximately 0·1 ml. of Tyrode solution. Each suspension was introduced separately into 5 ml. of embryo brei medium, and the resulting cultures were incubated as usual. After 1 day *B. pluton* organisms could be seen in large numbers in three of the inoculated tubes, but there was no sign of increase. The remaining tube of medium was contaminated by an organism which, on cultivation on other media, proved to be the so-called *Streptococcus apis*. Further examinations of the remaining tubes showed that *B. pluton* rapidly disappeared, and all three were apparently sterile bacteriologically after 30 days' incubation.

Exp. 3. The chorioallantoin membranes of three 13-day-old chicken embryos were slightly scratched, and were then inoculated by placing 0·1 ml. of a suspension containing approximately 4×10^6 *B. pluton* organisms on them. One membrane became contaminated by a torula. On the remaining two *B. pluton* refused to develop, the cells slowly disappearing. The chicken embryos continued to live and develop until they were about 18–19 days old, but died prior to the normal hatching time. *B. pluton* organisms could not be demonstrated either in the chorioallantoin membrane, or heart, liver, spleen or brain of the embryos.

Exp. 4. The whole stomachs of three apparently healthy larvae which contained *B. pluton* in apparently pure culture were placed on the chorioallantoin membranes of three 12-day chicken embryos. One embryo was also inoculated by driving a sharp needle through the chorioallantoin membrane. After 3 days' incubation the eggs were examined. One embryo (the one which had been inoculated directly) was dead and the whole contents of the egg were full of sporulating *B. alvei* organisms. The remaining two embryos were still alive and showed no *B. pluton* cells. Subsequently, one embryo died about 2 days prior to the normal time of emergence apparently free from bacteria, while the other matured normally and emerged from the inoculated egg.

Exp. 5. A 9-day-old embryo was inoculated directly through the chorioallantoin membrane with approximately 1×10^6 *B. pluton* organisms in 1 ml. of liquid. Examinations of the fluid beneath the chorioallantoin membrane and of the scrapings from the membrane itself, carried out at intervals during the 8 days following inoculation, showed that the bacterial cells rapidly disappeared.

Exp. 6. The chorioallantoin membrane of a 7-day-old embryo was inoculated with the greater portion of the stomach of a larva recently affected with European foul brood and in which *B. pluton* was present in apparently pure culture. The remaining portion was streaked on to the surface of a tube of beef digest brood filtrate agar, and no bacterial growth resulted after 1 week's incubation. The inoculated embryo was watched and was still alive after 10 days' incubation. Smears made from the chorioallantoin membrane and from the organs of the 17-day-old embryo showed no *B. pluton* organisms or other bacteria.

The results of the above experiments show that *B. pluton* will not grow on the minced tissues of the chicken embryo, on its chorioallantoin membrane or even in the embryo itself.

(ii) *Inoculation of individual larvae with* B. pluton

Suspensions of *B. pluton* in either Tyrode solution or phosphate buffer were prepared from the stomachs of larvae recently affected with European foul brood by the technique already described. These were prepared and suitably diluted with either Tyrode solution or phosphate buffer on the same day as the inoculations were made. Two or three comb nuclei of hybrid bees prepared as in previous experiments (Tarr, 1936) were used for the inoculations.

Exp. 1. A suspension was prepared containing approximately 40×10^6 *B. pluton* organisms per ml. of phosphate buffer. This suspension was tested for purity as follows: 0·5 ml. was inoculated on to the surface of a tube of beef digest brood filtrate agar; no growth resulted after the culture was incubated for 3 weeks. 0·5 ml. was placed on the chorioallantoin membrane of a 10-day chicken embryo. The embryo developed normally until a few days prior to the time of hatching. When examined *B. pluton* could not be detected in any of its organs.

On 2 July a comb containing chiefly eggs and young larvae was removed from a healthy nucleus and three groups of 100 young larvae about 1 day old in adjacent rows of cells were fed 0·001 ml. portions of the above suspension, or of dilutions prepared from it, so that each larva received approximately 40,000, 4000 or 400 *B. pluton* organisms respectively. The infective material was placed in the brood food surrounding the larvae. The comb was returned to the colony and larvae were removed at random from the inoculated area 2 and 3 days after inoculation, and their intestines examined microscopically for *B. pluton*. The results are given in Table I.

Table I. *Results of oral inoculation of individual larvae with* B. pluton

Approximate no. of *B. pluton* organisms fed	No. of larvae showing *B. pluton* cells in their stomachs			
	2 days after inoculation		3 days after inoculation	
	No. of larvae examined	No. showing *B. pluton*	No. of larvae examined	No. showing *B. pluton*
40,000	3	2	12	11
4,000	3	2	12	10
400	3	0	12	1
Controls (no *B. pluton*)	3	0	12	0

Most of the larvae which were infected had very large numbers of *B. pluton* organisms in their digestive tracts, and examination of the colony 3 weeks after inoculation showed that European foul brood had spread rapidly, a very large proportion of the larvae being affected.

Exp. 2. A suspension containing approximately 23×10^6 *B. pluton* organisms per ml. of Tyrode solution was prepared. 0·5 ml. of this suspension was injected through the chorioallantoin membrane into a 6-day embryo, and this was apparently sterile after 5 days' incubation and still alive. 1 ml. produced no growth when inoculated into 15 ml. of liquid beef digest brood filtrate agar which was allowed to solidify and then incubated.

On 13 July three different nuclei were selected and fifty young larvae about 1–2 days old in each were fed 0·001 ml. portions of the above suspension, or dilutions prepared from it, so that individual larvae received either 23,000, 2300 or 230 cells of *B. pluton*. Three days after inoculation ten of the inoculated larvae were removed at random from each nucleus and were examined microscopically. Seven of those which had received approximately 23,000 *B. pluton* organisms were affected with European foul brood, three of those which had received 2300 bacteria were similarly affected, but none of those fed 230 organisms had contracted the disease. Subsequent examinations showed that European foul brood spread rapidly in the two nuclei in which the larvae had been most heavily inoculated but did not occur during the remainder of the brood rearing season in the other nucleus.

The above experiments show that young larvae readily contract European foul brood when inoculated with relatively small doses of *B. pluton* organisms which are unable to produce growth on embryo medium or on beef digest brood filtrate agar.

(iii) *The development of* B. pluton *in the bee larva*

White (1920) stated that "Growth of *B. pluton* in the infected larva begins close to the surface of the peritrophic membrane in contact with the food of the larva. As growth continues the mass extends towards the lumen of the peritrophic sac finally filling it more or less completely. The growth does not always take place uniformly along the peritrophic membrane, nor does it extend beyond it, but is enclosed within the sac, the tissues of the larvae not being reached. The multiplication of the organism after the death of the host, if, indeed, it takes place at all, is limited." Morgenthaler (1930) has verified these statements, at least in part, and similar results have been obtained by the writer. These are given below.

Larvae of all different ages and stages of European foul brood were removed from affected bee colonies. They were immediately fixed in Carnoy's fluid, later imbedded in paraffin wax and sections cut. The sections were stained with Ehrlich's haematoxylin, Gram's stain and eosin. It was found that the tissues stained well by haemotoxylin or

eosin, while the *B. pluton* organisms, being Gram positive, were best stained by this stain. A detailed description of the photomicrographs prepared from these sections (Pls. XXXIII–XXXV, figs. 1–17), is appended. These photographs show the distribution of *B. pluton* in its host at different stages in the disease.

DISCUSSION

The fact that *B. pluton* has refused to grow on any medium other than that afforded by the food mass within the peritrophic sac of the developing bee larva indicates that this organism is a strict parasite. Previous attempts to cultivate this species in starved larvae (Tarr, 1937) also met with failure, but it is possible that it will develop in larvae reared in the laboratory by a method similar to that described by von Rhein (1933). Trager (1936) claims that he has grown the virus of Grasserie in tissue cultures of silkworm ovaries, and it would seem worth while attempting to cultivate *B. pluton* on tissue cultures of bee larvae. It may prove impossible to grow this species, but further attempts should be made. It is of interest that relatively small numbers of *B. pluton* organisms are able to initiate European foul brood in young larvae when mixed with the food mass surrounding them. In this respect European foul brood differs radically from American foul brood, where inoculation of individual larvae with large doses of the causal organism has always failed to produce the disease (Tarr, 1938 *b*). It has been shown that European foul brood is purely a disease of the digestive tract of the larva, *B. pluton* growing only in the food mass within the peritrophic membrane and probably killing its host before it, or any of the other bacteria associated with the disease, succeed in invading the body tissues. Whether this also applies to larvae affected with American foul brood remains to be determined.

SUMMARY

B. pluton will not grow on the chorioallantoin membrane of the developing chicken embryo or on embryo brei medium made from minced embryos.

European foul brood is a purely intestinal infection of the bee larva, the causal organism being localized in the food mass within the peritrophic membrane.

Young larvae are readily infected with European foul brood by mixing relatively small numbers of *B. pluton* organisms, obtained from recently affected larvae, with the brood food surrounding them.

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EXPLANATION OF PLATES XXXIII–XXXV

The following symbols have been used, most of which are the same as those employed by Nelson (1924). *An*, Anus. *B.m.*, Bacterial mass. (Food matter plus bacteria in badly affected larvae.) *B.p.*, *Bacillus pluton*. *M.Int.*, Mid intestine. *Mth*, Mouth. *Oe.*, Oesophagus. *P.gr.*, Pollen grain. *P.mb.*, Peritrophic membrane. *qq.*, Food matter in mid intestine. *Slk.Gl.*, Silk gland. *Str.Bor.*, Striated border of epithelium of mid intestine.

PLATE XXXIII

Fig. 1. × 37·5. Transverse section through a larva 2–3 days old recently affected with European foul brood. *B. pluton* organisms occur only between the peritrophic membrane and food matter. The uneven distribution of *B. pluton* along the peritrophic membrane is shown by the fact that this organism was present in only a few of the sections made from this larva. The peculiar "granular" structure of the so-called "peritrophic membrane" was described by Nelson (1924) and can be seen in figs. 1–9. It is not known whether the membrane alters when the larvae are "weaned" from brood food to honey and pollen, nor whether it is of chitinous nature, as in adult bees and many other insects.

Fig. 2. × 37·5. Transverse section through another portion of the larva used in making Fig. 1, only showing somewhat heavier invasion by *B. pluton*. This figure also serves to show the uneven distribution of *B. pluton*.

Fig. 3. × 60. A portion of Fig. 2, somewhat enlarged, showing the clear line of demarcation between the *B. pluton* organisms and the peritrophic membrane.

Fig. 4. × 300. Part of Fig. 3, greatly enlarged, showing the penetration of *B. pluton* organisms into the food mass but not into the peritrophic membrane.

Fig. 5. × 12. Larva about 3 days old, at the time of weaning from brood food to honey and pollen, recently affected with European foul brood. The longitudinal section shows the sharp demarcation between peritrophic membrane and food mass as well as the irregularly distributed groups of *B. pluton* between these.

Fig. 6. × 60. An enlarged portion of the longitudinal section used in making Fig. 5. Groups of *B. pluton* are shown between the food matter and peritrophic membrane.

Fig 1

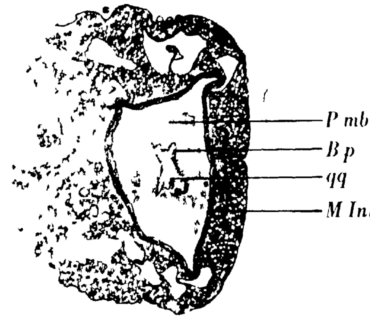

Fig 2

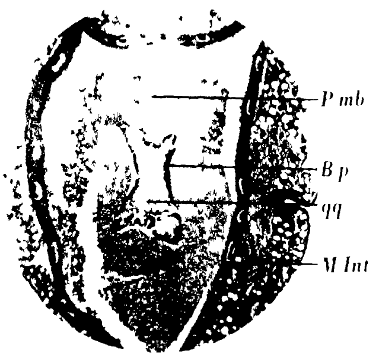

Fig 3

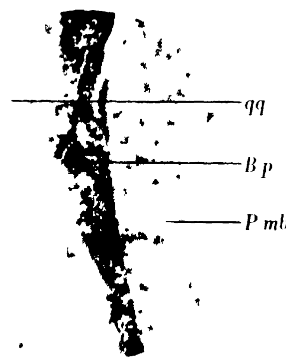

Fig 4

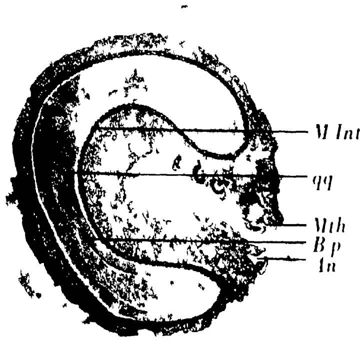

Fig 5

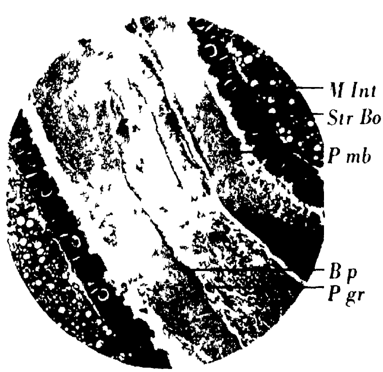

Fig 6

FARR Studies on European Foul Brood of Bees (pp 815 821)

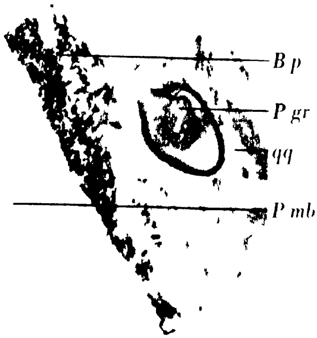

Fig 7

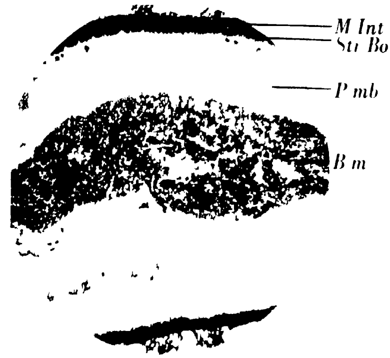

Fig 8

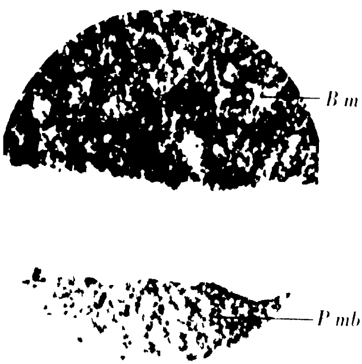

Fig 9

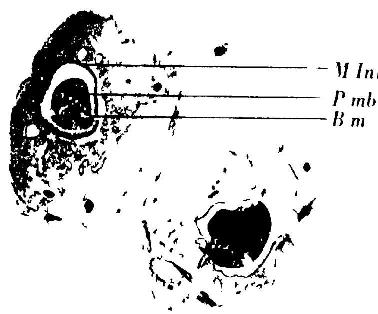

Fig 10

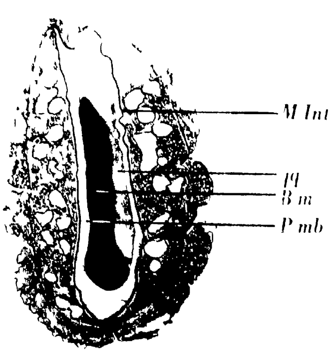

Fig 11

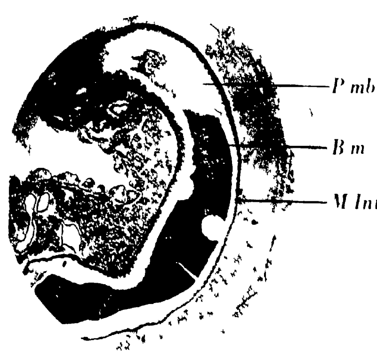

Fig 12

Fig. 13

Fig. 14.

Fig. 15

Fig. 16

Fig. 17.

TARR.—Studies on European Foul Brood of Bees (pp. 815 821)

PLATE XXXIV

Fig. 7. ×600. A greatly enlarged portion from the section used in making Fig. 6. *B. pluton* is shown penetrating into the food matter but not into the peritrophic membrane.

Fig. 8. ×60. Transverse section of a larva from 3 to 4 days old in which European foul brood has reached a fairly advanced stage. The whole food mass has been penetrated by *B. pluton*, but the peritrophic membrane is still apparently intact.

Fig. 9. ×600. Enlarged portion of the section used in making Fig. 8. The whole food matter has been invaded by *B. pluton* while the peritrophic membrane is still sharply demarcated from the infected area.

Fig. 10. ×12. A transverse section through a coiled larva about 4 days old very badly infected with European foul brood and probably dead. The whole mass of food has become practically a sac of bacteria, but these have not yet penetrated the body cavity of the larva. The peritrophic membrane has nearly disappeared. The epithelium of the intestine has apparently disintegrated.

Fig. 11. ×12. A semi-transverse section through the larva used in making Fig. 10. The bacteria are not evenly distributed through the food mass. The peritrophic membrane has nearly disappeared and the epithelium of the mid intestine has disintegrated.

Fig. 12. ×12. Longitudinal section through a 4–5 day old larva badly affected with European foul brood and probably dead. The whole food mass is full of bacteria, the peritrophic membrane has practically disintegrated, and the intestinal epithelium has lost its characteristic structure.

PLATE XXXV

Fig. 13. ×7·5. A larva about 5 days old very badly affected with European foul brood and stretched out ready for sealing. The food mass has been entirely filled with bacteria and has sunk to form a bacterial sac at the rectal end of the larva. The peritrophic membrane has almost disappeared, and the intestinal epithelium is disintegrating. An affected silk gland is shown; the causal organism evidently penetrates this gland via the opening into the oesophagus.

Fig. 14. ×7·5. A section similar to that used in making Fig. 13 and from the same larva. The oesophagus and silk glands have been invaded by *B. pluton*.

Fig. 15. ×60. An enlarged portion from Fig. 14 showing the food mass invaded by bacteria and full of pollen grains. The peritrophic membrane has practically disappeared, and the mid intestinal epithelium is disintegrating.

Fig. 16. ×600. A portion of Fig. 15 greatly enlarged. The food mass has been entirely invaded by *B. pluton* and other bacteria (secondary invaders), and only a thin layer of peritrophic membrane remains, but this still prevents the bacteria from penetrating the intestinal epithelium.

Fig. 17. ×600. A section through a 4–5 day old larva very badly affected with European foul brood. *B. pluton* and secondary invaders are seen penetrating the remains of the peritrophic membrane preparatory to invading the body cavity of the larva, which is probably dead.

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ON THE CHANGES IN CHEMICAL COMPOSITION ASSOCIATED WITH LARVAL DEVELOPMENT IN THE SHEEP BLOWFLY

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(With 5 Text-figures)

CONTENTS

Introduction

While the study of the biochemical changes associated with insect development has attracted an increasing number of workers during recent years, most attention has been paid to changes taking place during metamorphosis, and those associated with larval development, though of greater interest in the study of growth in general, have been somewhat neglected. The paucity of chemical information on the early larval stages of insects is, probably, partly due to the difficulties of obtaining and handling the necessary amounts of material; for example, in the course of the present work some 28,000 newly hatched larvae were used in two fatty acid estimations alone.

The sheep blowfly, *Lucilia sericata* Mg. (Diptera, Muscidae), suggested itself as a subject by reason of its short life-cycle, and the relative ease with which a permanent culture can be maintained for the supply of the necessary numbers of eggs throughout the year; it has the additional advantage of having been the subject of a considerable amount of work

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on related problems, such as the nutritional requirements of the larvae (Hobson, 1933, 1935*a* and *b*), and the chemical changes during metamorphosis (Evans, 1932). Muscid larvae were also the subject of Weinland's classical work on the synthesis of fat from protein (1909), which, despite the work of later investigators (Nishikata, 1922; Yuill & Craig, 1937), has still to be confirmed with sterile material and using satisfactory analytical methods.

MATERIAL AND METHODS

Egg material for analytical work was used within 1–3 hours of oviposition, to avoid discrepancies due to the rapid embryonic development. Newly hatched larvae were obtained by keeping eggs in saturated air at 25° C. for about 18 hours; the larvae were allowed to wander from the eggs on to filter paper, from which they could readily be collected on the point of a seeker. Their inability to resist desiccation renders rapid handling essential, and their removal in sufficient quantity direct from a glass surface was found impracticable. For later stages the larvae were reared on minced lean beef at 25° C. Body weight was found to be a better measure of state of development than age, and mean weights were determined, either for each whole batch or, with smaller larvae, for half a dozen or so samples of 20–50 larvae each. When full grown the larvae were allowed to migrate into clean dry sand; "prepupae" were used within a day of leaving the meat, after the crop had been emptied. All stages except the unfed newly hatched larvae were washed with several changes of distilled water and dried on filter paper before use; this treatment was found to be without effect on the moisture content.

Total fatty acids and unsaponifiable matter were estimated by a saponification method, as used by Evans (1932, 1934); mean molecular weight determinations, however, showed his second saponification to be unnecessary if the number of washings is adequate. Iodine values were determined by the method of Rosenmund & Kuhnhenn (1923). Total nitrogen was estimated by the micro-Kjeldahl method, which could be used for individual full-grown larvae. Total glucose, after acid hydrolysis, was determined by the Hagedorn-Jensen method, zinc hydroxide being used as a protein precipitant.

RESULTS AND DISCUSSION

On account of the 700-fold increase in weight during larval development the percentage of each constituent was considered the most useful figure for plotting. The best measure of the state of development of the larva was considered to be provided by body weight, which was plotted logarithmically, since for a considerable part of development there is a linear relationship between log. weight and age (unpublished results).

The limits of the three larval instars are indicated by arrows, and a rough time-scale for the temperature used (25° C.) is also given with each figure. Each point represents a single determination; the crosses apply to larvae, while the circles to the right of each curve apply to the first

day of the prepupal stage, and corresponding values for the newly laid egg are given for comparison by the circles on the left.

(i) *Moisture*

The newly laid eggs contain about 79 % water, distinctly less than the larvae at hatching (83 %) (Fig. 1); this is probably due to the low moisture content of the egg-shell. From hatching until the middle of the third instar the moisture content falls gradually to about 80 %, and then shows a very marked decrease to just under 70 % in the prepupae. Progressive dehydration is of very general occurrence in growing organisms, and the

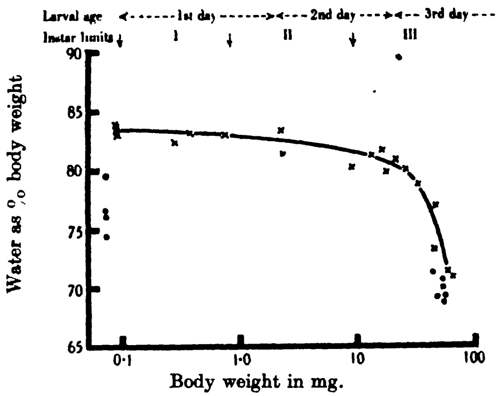

Fig. 1. Changes in moisture content.

accompanying decrease in the extent of dispersion and hydration of the tissué proteins is considered likely to be one factor in the usual decline in metabolic rate with age (Jordan Lloyd, 1932). There is certainly a considerable similarity between the present moisture content results and those found for respiratory rate (to be reported in detail in a later paper); the oxygen uptake falls only from 7 c.c./g./hr. at hatching to about 5·5 c.c./g./hr. at the end of the second instar, while during the third the rate falls to 2 c.c./g./hr. in the full-grown larva and less than 1 in the prepupa. However, the accumulation of inert reserve material during the third instar is likely to be another factor concerned in the declining metabolic rate.

The final rapid fall in moisture content is probably associated with the fact that the whole of larval life is spent in the saturated environment provided by the food, while after feeding ceases the larva leaves the meat and migrates to the much drier surroundings to pupate. The change is

not, however, passive desiccation, since it certainly begins before the meat is quitted. Furthermore, a decrease in moisture content has been found in a number of other insects to accompany an increase in resistance to low temperatures (Buxton, 1932), and the prepupa is the stage in which this species overwinters.

Tangl (1909) gave a moisture content of 69% for larvae of *Ophyra*, the corpse fly, probably full grown, and Tomita & Kumon (1936) recorded 77% for 15 mg. larvae (probably early third instar) of the flesh-fly *Sarcophaga*. Yuill & Craig (1937) give some rather variable figures for *Lucilia sericata* showing a fall from about 83% at the end of the second instar to about 70% in the full-grown larvae. These data all agree substantially with the present results, but Rudolfs' figures (1926*a*) for the tent caterpillar *Malacosoma* differ considerably, perhaps associated with the more exposed environment of the leaf-eating caterpillars; they showed an increase from less than 40% at hatching to 84% during the latter part of larval life, followed however by a fall before pupation as in *Lucilia*. Larvae of the wax-moth, *Galleria*, living in the probably more humid atmosphere of the bee-hive, behave more like *Lucilia*, showing a fall from 75% water at 10 mg. body weight to 56% at 200 mg. (Teissier, 1931).

(ii) *Fats*

The eggs when laid have a fatty acid content of 17–19% dry weight, falling to 9–13% in the newly hatched larvae. From these figures, together with further evidence from work on the respiration of the eggs, to be published in a later paper, fat appears to be the main energy source during embryonic development. This is also the case in such other insects as have been investigated—the silkworm *Bombyx* (see Needham, 1931), the tent-caterpillar, *Malacosoma* (Rudolfs, 1929), and the grasshopper *Melanoplus* (Slifer, 1930; Boell, 1935)—and would of course be expected from the fact that the class as a whole produces typical cleidoic eggs.

The proportion of fat probably shows little change till the end of the second instar, when a rapid accumulation begins, continuing to the end of larval life, when a maximum of nearly 30% is reached, falling to about 27% within the first day of leaving the meat (Fig. 2). This agrees closely with the conclusions of Davies & Hobson (1935); they demonstrated a rapid increase in the resistance of the larvae to starvation (in saturated air) between the early second and early third instars, and suggested that the storage of reserve material probably began at about this stage. There are a number of records of increasing fat content during larval life, e.g.

Rudolfs (1926*a*) showed an increase from 1% at hatching to 29% at pupation in *Malacosoma*, and Teissier (1931) found the fat content of the meal-worm *Tenebrio* to increase as the 1·13th power of the body weight during the latter part of larval life.

In nearly all such work, however, fat has been estimated as crude ether extract; a Soxhlet extraction of the prepupae was carried out for comparison with the present saponification results. 22·5% crude fat was obtained, corresponding to 20% fatty acids, as compared with the 25–27·5% obtained directly. Discrepancies in the earlier stages are likely to

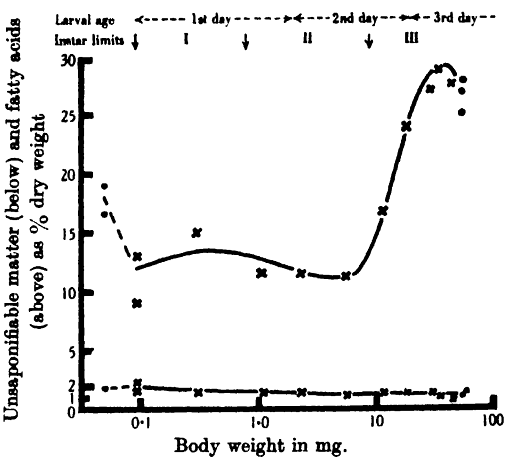

Fig. 2. Changes in fats.

be considerably greater on account of the smaller size of the larvae, the smaller proportion of fat present, and the fact that much of the vital fat is probably in the form of complex lipoids not readily extracted with ether. This point is illustrated by the recently published work of Yuill & Craig (1937), on the larval fat metabolism in this species. They found 6% fat (crude ether extract) in the earliest stage studied (late second instar), while the present results give 14% (glycerides plus unsaponifiable) at this stage. The discrepancy decreases as the fat content rises; Yuill & Craig find a maximum of 30%, while the saponification method gives one of 31%. The data of these authors confirm the rapid storage of fat during the third instar which was found in the present investigation, but it is unfortunate that out of their thirty-seven fat estimations on larvae, none was done on first instar material, three during the second, and all the remainder during the third.

The 11% or so of fatty acids present during the first two instars in this material is probably mainly "élément constant" or "vital fat", though some of it may represent hydroxy-fatty acids produced by the deamination of the food proteins, which are possibly the larva's main energy source (Brown, 1936). Evans (1932) found an acetyl value of zero during metamorphosis, indicating an absence of hydroxy-acids at that time, but no information is available for the larval period. It is also possible that the very high metabolic rate of the larvae, presumably involving great activity at cell surface membranes, may be associated with a relatively high "élément constant".

With two exceptions, all the iodine values obtained for larvae lie between 67 and 73, and the first exception, a low value obtained on a very small sample of fatty acids from newly hatched larvae, seems almost certainly aberrant. In the case of the second, however, a value of 92 was obtained at the beginning of the third instar; duplicate determinations on the same batch of fatty acids differed by less than one unit, and so this value may possibly be significant, particularly as it applies to the stage when the accumulation of reserve fat is just beginning. It therefore seems likely that the degree of unsaturation of the fatty acids does not vary appreciably during larval life, with the possible exception of an increase when the accumulation of reserve fat begins. During the latter half of larval life Yuill & Craig also found a steady iodine value, with the exception of a slight fall near the end on the fish diet (possibly representing an increase in the proportion of fat synthesized from protein to fat derived directly from the food, in the reserves being laid down). These results do not agree with the usual idea of a highly unsaturated "élément constant", and further evidence that this idea is not applicable to *Lucilia* is provided by Evans's observation that during metamorphosis the saturated acids remain practically constant, only the unsaturated ones being consumed. The possible significance of the low iodine values found for the eggs (34–44) is discussed in a later section.

Another factor likely to influence the degree of unsaturation of the reserve fat is the environmental temperature; similar cultures were set up at 15, 25 and 35° C., and batches of prepupae were saponified within 24 hours of leaving the food in each case. Iodine values of 75·8, 72·7 and 71·0 were recorded respectively for the three temperatures, showing differences which are very small compared with those found, e.g. by Terroine *et al.* (1927), for certain fungi and bacteria; further, even the differences observed may be explained in part by the selective utilization of unsaturated fatty acids (Evans, 1932), which begins on leaving the

meat, and which will certainly occur at a rate varying with the temperature.

Yuill & Craig, however, have demonstrated the large qualitative effect of the fat in the diet on the reserve fat laid down; on a fish diet containing an oil of iodine value 113, the larval fat gave an iodine value of 120–140, while on a diet containing butter as the source of fat (I.V. 30), the iodine value of the larval fat was 60. These results suggest that while, as Weinland (1909) showed, the bulk of the reserve fat is probably synthesized from protein, yet preformed fat in the diet is also incorporated in the larval reserves.

The evidence so far available thus suggests that of the various factors likely to influence the degree of unsaturation of the fat in the larva of *Lucilia*, the nature of the food fat is easily the most important; the state of development appears to have no effect, except for the possible rise at the beginning of the third instar; and, at any rate on a diet containing preformed fat (such as is likely to be encountered in nature), the effect of environmental temperature is very slight.

Samples of fatty acids from the prepupae gave mean molecular weights of 272–290, in satisfactory agreement with Evans's figures of 270–287. Both iodine value determinations for this stage gave 72·7 (at 25° C.); Evans found 79–96 at 17° C.

Though the figures for unsaponifiable matter should not perhaps be taken too seriously, on account of the very small amounts of material concerned (usually less than 10 mg.), the general trend of the figures indicates a fall, from about 2% at hatching to about 1% in the prepupa. This fraction is of particular interest in view of Hobson's demonstration (1935) that cholesterol is the only fat-soluble substance essential for the larval growth of *Lucilia*.

(iii) *Ash*

As pointed out by Teissier (1931), the ash may often be considered as a very rough measure of the protoplasm present, consisting as it does mainly of the mineral salts which form an essential constituent of living matter: reserve materials and chitin are usually practically ash-free. In some cases this generalization is inapplicable on account of the large amounts of inert skeletal or excretory material such as calcium salts and silica, but the results of Tomita & Kumon (1936) for larvae of another blowfly, *Sarcophaga*, showing that 84% of the total ash consists of the phosphates and chlorides of sodium and potassium, suggest that it may safely be applied here.

The newly laid egg contains about 7 % ash, while the larva has about 10 % at hatching; the observed consumption of fat during embryonic development only accounts for about one-fifth of this difference, suggesting a low ash content for the egg-shell.

The percentage of ash falls steadily during larval life, most rapidly during the latter part of the third instar (Fig. 3). In part this is explained by the storage of fat, but this is not the entire and probably not even the chief cause, for when expressed in terms of fat-free dry matter ash still falls from more than 11 % at hatching to 5 % in the prepupa. This demonstrates a considerable accumulation of materials of low ash content in addition to fat, and there is other evidence, discussed later, that much of this may be reserve protein.

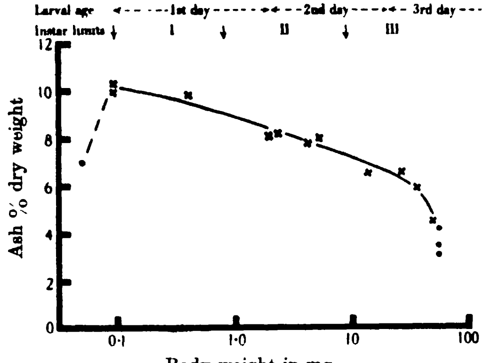

Fig. 3. Changes in ash.

Tomita & Kumon recorded 7 % of ash for *Sarcophaga* larvae at about the beginning of the third instar, in close agreement with the present results; Teissier also found a considerable decrease in ash during larval life, from 5 % at 10 mg. to 1 % at 200 mg. body weight in *Galleria*.

(iv) *Nitrogen*

The results of Evans (1932) for this species show that 82 % of the total nitrogen is in the form of protein at the beginning of the prepupal period; furthermore Brown (1936) has shown that the total ammonia excreted during larval life in *Calliphora* on a meat diet only amounts to about 2·3 % of the body weight at pupation, and hence the amount of ammonia present at any given period will be small compared with the amount of body protein. It does not, therefore, seem unreasonable to

make the usual assumption of regarding the total nitrogen of the larva as a rough measure of the protein present.

The eggs gave a weighted mean of 12·5% nitrogen and the newly hatched larvae one of 12·7%, while the egg-shell material left after hatching gave 11·4–11·6%. The chorion gave strongly positive Millon and xanthoproteic reactions, but the nitrogen content found indicates non-protein constituents as well. The newly laid egg was completely soluble in potash under the conditions prescribed by Campbell (1929), indicating the absence of chitin at this stage, though the cuticle of the newly hatched larvae gave a strongly positive van Wisselingh reaction.

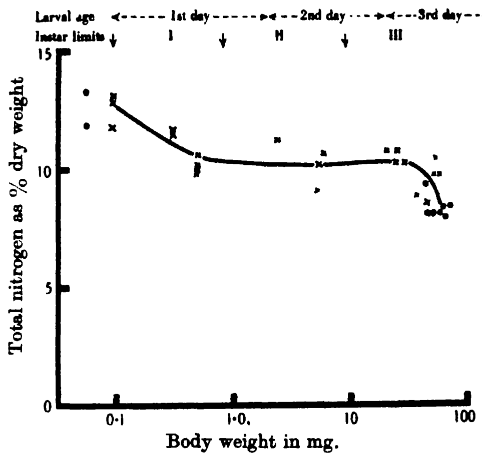

Fig. 4. Changes in nitrogen

Molisch's test, though positive for the contents of the undeveloped egg, was completely negative for the chorion, showing the absence of other polysaccharides and of mucoproteins. Another possibility is the presence of fats or waxes in the shell; the loss of fatty acids calculated from the mean values found for the newly laid egg and the newly hatched larva is actually about 10% greater than can be accounted for by the whole of the oxygen uptake during embryonic development, and, while on account of the variability of the analytical data this fact is in itself of little significance, yet, taking into account also the low iodine values found for the eggs, the evidence available seems to point to the presence of saturated fatty acids in addition to protein in the chorion.

The nitrogen content of the larvae falls from about 12·5% at hatching to about 10% by the end of the first instar (Fig. 4), and appears to remain

at about that figure till practically the end of larval life, when a further fall occurs, to about 8·5% in the prepupa; the latter change will represent mainly the nitrogen excreted after feeding ceases. Similarly in *Malacosoma* Rudolfs found a fall from 15% at hatching to a minimum of 8·5% in late larval life. Owing, however, to the considerable changes which are taking place in other constituents, notably fat, Table I, constructed from the present results, is perhaps more instructive than the percentage figures.

Table I. *Nitrogen/ash ratios of larvae at various stages*

Stage	Body weight in mg.	Nitrogen/ash
Hatching	0·09	1·3
Mid 1st instar	0·25	1·2
End 1st instar	0·80	1·1
Mid 2nd instar	3	1·3
End 2nd instar	9	1·4
Early 3rd instar	20	1·5
Late 3rd instar	40	1·7
Prepupa	55	2·3

It has previously been pointed out that most of the ash probably represents the mineral salts which are an essential constituent of protoplasm, and that the greater part of the nitrogen is present as protein. Since the composition of protoplasm is unlikely to vary to the extent shown by Table I, it is suggested that at the end of larval life something approaching half the protein present represents relatively ash-free reserve material. There is some histological evidence also for this storage of protein; the cells of the pupal fat-body contain numerous semi-solid granules giving positive biuret and xanthoproteic reactions (Evans, 1935). It seems likely that these will have been formed by precipitation at their isoelectric point, and hence that their ash content will be at a minimum.

Table I also suggests the disappearance of a certain amount of reserve protein during the first instar; reserves of some sort must almost certainly be present even at hatching, since under saturated conditions the unfed larvae can survive for 21 hours even at 37° C. (Davies & Hobson, 1935). Some utilization of reserves will probably occur normally while the larvae are establishing themselves on the meat.

As the food of the larvae consists very largely of protein, it would be expected that the error, present in all these experiments, due to the presence of undigested food in the crop, would be most serious in the nitrogen estimations. Three determinations of the nitrogen content of gorged full-grown larvae gave a mean of 2·64% (fresh weight), as compared with 2·49% after the crop was emptied. It is, therefore, probable that the

whole of the curve given, with the exception of the points for the egg, the newly hatched larva, and the prepupa, is something like 10% too high; this correction would accentuate the change in the nitrogen/ash ratio recorded above.

The presence of a variable amount of food protein in the crop is probably also partly the cause of the nitrogen figures showing more variability than the other determinations, though the small size of the samples used will also give greater prominence to individual variation.

(v) *Carbohydrate*

As a contrast to the results obtained for fats, the egg was found to contain a slightly lower percentage of carbohydrate than the newly hatched larva. During larval life the percentage of total glucose falls from about 10 to 7% (Fig. 5). If, however, fat is deducted and a similar

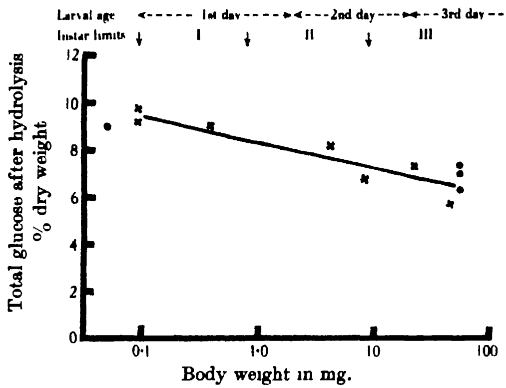

Fig. 5. Changes in carbohydrate

rough correction, based on the nitrogen/ash figures, applied for reserve protein, glucose as a percentage of the remaining dry matter falls from 12 to 9·5% during the first instar, remains at about 9% during the second, and finally increases to 14% in the prepupa. If the validity of this treatment of the results be accepted, it would appear that reserve carbohydrate is utilized in addition to protein, while the larvae are becoming established on their food medium, and that a further reserve of carbohydrate is laid down during the third instar.

The recent work of Kuwana (1937), on the body fluid of *Bombyx*, suggests however that some caution is necessary in the interpretation of results of this kind, particularly in expressing reducing power in terms of

glucose; for example, the amount of glucose present in the body fluid of the silkworm as free fermentable reducing agent is negligible, except for a temporary rise after feeding to about one-eighth of the total reducing power of the deproteinized filtrate. Acid hydrolysis, however, roughly doubles the total reducing power; practically the whole of this increase was shown to be fermentable, and at least half of it could be attributed with certainty to glycogen. Kuwana followed the total reducing power, both before and after hydrolysis, from the beginning of the fourth instar; in both cases maxima were obtained just before the fourth moult and just before pupation. Glycogen, estimated directly, remained at a steady value during the larval stages and rose to a maximum of about 2·5 times this value at pupation.

Considering glycogen alone, Rudolfs found a fall from 2·8 to 0·2% during larval life in *Malacosoma*, the actual amount present, however, increasing some thirteen times over this period. In the moth *Dictyoploca* water-soluble reducing sugars increased during the first few days of larval life, but decreased rapidly thereafter; glycogen also was only found in traces in the pupa (Shinoda, 1926). In the bot-fly *Gastrophilus* much glycogen is formed in early life, but a large proportion is later transformed to fat (Kemnitz, 1914; Blanchard & Dinulescu, 1932). The fully grown bee larva on the other hand contains considerably more glycogen than fat (Straus, 1911).

SUMMARY

The most marked change in composition during embryonic development is the disappearance of fat typical of cleidoic eggs.

The chorion probably consists of protein together with some nitrogen-poor constituent, perhaps a saturated fat or wax; chitin and other carbohydrates are absent, and ash and moisture contents are low.

No great changes in composition occur during the first two larval instars, with the exception of a possible utilization of some reserve protein and carbohydrate while the larvae are establishing themselves on the meat.

During the third and last instar, however, a considerable reduction in moisture content occurs, and an extensive storage of fat takes place, beginning at about the stage at which previous work had shown a marked increase in resistance to starvation. There is also evidence for the accumulation of a large bulk of reserve protein and a smaller amount of reserve carbohydrate during this instar.

The most important factor influencing the degree of unsaturation of the larval fat appears to be the nature of the food fat; iodine values show little variation with the state of development, and preliminary observations suggest that the influence of the environmental temperature is small.

I wish to record my sincere thanks to Prof. P. A. Buxton for his active interest in this work, and to Dr V. B. Wigglesworth for helpful advice and valuable suggestions. I am also indebted to the Agricultural Research Council for the award of a Research Scholarship which has entirely financed this investigation.

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THE USE OF PROTECTIVE FILMS OF INSECTICIDE IN THE CONTROL OF INDOOR INSECTS, WITH SPECIAL REFERENCE TO *PLODIA INTERPUNCTELLA* Hb. AND *EPHESTIA ELUTELLA* Hb.

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CONTENTS

INTRODUCTION

INSECTS belonging to the genera *Ephestia* and *Plodia* are among the principal pests of stored products in this country and elsewhere. The two most important species are probably *Ephestia elutella* Hb., the cacao moth, which is one of the chief pests of cacao, dried fruits and tobacco, and *Plodia interpunctella* Hb., the Indian meal moth, which is a very important pest of dried fruits, nuts and cereals.

One of the noteworthy points in the biology of these two species is that the majority of the fully grown larvae leave their food and wander away to pupate in some crack or crevice. In this way a large number

reach the fabric of the warehouse storing the infested goods. The moths which result from these larvae will infest any susceptible goods that are in the warehouse when they emerge.

It is evident that for the purpose of control there are two sources of infestation. The first is the insects which arrive from outside the warehouse, either by way of infested goods or by other means; the second is from inside the warehouse, due to the moths emerging from the premises.

With a few possible exceptions, goods can be freed from infestation prior to storage, by fumigation in suitable chambers or by other means. This treatment will ensure that no infestation is imported with the goods. The clean goods can then be taken to the warehouse. The problem of protecting the clean goods from reinfestation by insects from the warehouse or elsewhere, is more difficult.

One method of cleaning the warehouse is to leave it empty throughout the summer months, so that there is no food upon which moths emerging from the premises can lay their eggs. Usually this method is not economically possible. A second method is that of fumigating the premises, but unless the warehouse has no cracks and crevices and can be made airtight, it is not certain to give good results. An account of a series of fumigations with hydrogen cyanide and ethylene oxide has been given (Potter, 1937), which shows the difficulty of killing the full grown larvae of *E. elutella* and *P. interpunctella* when they are in crevices in the fabric of the warehouse. The most serious objection to these two methods is that they do not guard against reinfestation during the summer months when the moths are flying. It appears from observation, that infestation by moths which fly in from outside is very likely to occur. A control method was devised to overcome these difficulties. It was essentially a preventive method and consisted of atomizing a solution of pyrethrins in white oil to form a mist in the warehouse. The mist was put up in the warehouse every night throughout the period of moth emergence, so that no moths were able to reach and infest the stored goods. This method was first used for the protection of dried fruits in London, where it was completely successful; the apparatus and technique has been described (Potter, 1935). It was then introduced in Bristol, Liverpool and Glasgow, and warehouses in each of these towns were rid of infestation in a year.

The objection to this method is the expense, since the cost of apparatus, materials and labour to spray every night for about 3 months is heavy.

There were indications during the application of this method that its success was due to a protective film of insecticide which formed on all

exposed surfaces when the mist settled. Any moth settling on this film was incapacitated and finally killed. It was obvious that a study of the film might lead to a modification of the method which would be more economical. The method of forming a protective film of insecticide might also be of use in other branches of industrial and medical entomology.

This paper describes field and laboratory experiments which show that by a modification of the atomizing method previously described, a protective film can be formed which gives complete protection against *P. interpunctella* and *E. elutella* in the warehouse and is also very much more economical in apparatus and labour than the previous method. The experiments also indicate the possibilities of further investigation into the use of protective films of insecticide against other indoor pests.

(a) *The objects of the experiments*

(*a*) The production of a film of insecticide in the warehouse which will prevent moths of *E. elutella* and *P. interpunctella* which emerge from the fabric of the warehouse or which enter from outside from infesting the goods stored in the warehouse.

(*b*) A study of the effect of the film on the migrating larvae of these species, so that infestation from this source may be prevented or localized if it does occur.

(*c*) A study of the lasting properties of the film.

(*d*) A short preliminary study of the effect of the insecticidal film on other species of insect.

(b) *The insecticide*

The insecticide used throughout the experiments consisted of a solution of approximately 0·8% pyrethrins in a white oil of the following specification: sp.gr. 0·862; flashpoint closed, 320° F., flashpoint open, 335° F.; visc.redw. 1 at 70° F. 118 sec.; unsulphonated residue, 99·2% by vol.; pour test, −30° F., and sufficiently refined to be transparent, colourless and tasteless; this oil which resembles medicinal paraffin is marketed under the name of Shell oil 24210. This insecticide has no fire risk and has already been used on cacao, tobacco and dried fruits without having any deleterious effects.

Previous experiments (Potter, 1935) had shown that this insecticide is effective against the larvae of *E. elutella* and *P. interpunctella* when sprayed directly on to them, and to the adults when atomized in the form of a mist.

In the first three tests in the semi-field scale experiments and in the field scale trial the insecticide was made up by mixing one part of a commercial extract of pyrethrins with seven parts of Shell oil 24210. For the remainder of the tests, an extract of ground flowers with petroleum ether (B.P. below 40° F.) was standardized by the Seil method. This solvent was then removed and the pyrethrins taken up in Shell oil 24210 to make an 0·8% solution. It is thought that the heavy oil used as a carrier makes the formation of a lasting film possible, but no work has been done on the effect of different carriers. Further experiments have shown that other toxic agents, such as the organic thiocyanates, may be used instead of pyrethrins, but there is as yet insufficient knowledge of their effect.

(c) *Organization of the work*

The work was first carried out under semi-field conditions, using the commercial apparatus; a full-scale field trial was then made, which proved successful; finally, a more detailed investigation of the properties of the film was made in the laboratory under controlled conditions.

SEMI-FIELD EXPERIMENTS

These experiments were carried out at a London warehouse under the normal conditions of unheated storage. A film of insecticide was applied as evenly as possible to boards of Canadian hemlock, an absorbent wood used for making dried-fruit boxes. The film was applied with the atomizing gun which is used in practice and which has already been described and figured (Potter, 1935). The degree of atomization was standardized by timing out a given quantity of the insecticide at a known pressure.

In field experiments of this kind it is very difficult to measure what percentage of the material sprayed on to a surface actually adheres to form a film. Absorbent paper was sprayed under similar conditions to the test boards and, by weighing the paper before and after spraying, it was found that about 0·6 of the material sprayed adhered to form the film.

In the second experiment the box was covered with a glass lid so that the film was exposed to diffused light; in the rest of the experiments the films were kept in the dark. Films of pyrethrum insecticide kept in the dark would last longer than those exposed to light, but in general the interior of a warehouse is dim and would not cause rapid deterioration, and by having a glass lid an uncoated surface was presented which might affect the results of the experiment.

After the boards had been sprayed they were made up into a box with

the coated surfaces forming the interior of the box; the test insects were placed inside. All the experiments were made on fully grown larvae of *P. interpunctella* and *E. elutella*, chiefly on the former since they are more resistant to the insecticide used. The first two experiments were made in the winter and most of the larvae had spun cocoons from which they had to be removed. This treatment made them less resistant than the normal migrating larvae, as is shown by the later experiments, but the difference in resistance does not appear to be very great.

In practice, the essential property of the film is that it should kill the moths, but the larvae are very much more resistant than the moths, and a film which takes a long while to affect the larvae will kill the moths in a very short time. The fully grown larvae were chosen for these experiments because it was possible to obtain them in large numbers, because they are easier to handle and because, in a number of situations, it is desirable that the film should be lethal to the larvae as well as the moths. A preliminary experiment was made to ascertain whether a film of the insecticide could kill the larvae; the interior of a box was heavily coated with insecticide and 150 larvae of *P. interpunctella* and 75 larvae of *E. elutella* put in it. They were severely affected after a short exposure but were left for a week before removal: all the larvae subsequently died.

Having obtained definite evidence that the film could be toxic to the larvae, a further series of experiments was made under more controlled conditions.

The following data give the general conditions of the succeeding experiments: Width of box, 13 in.; length, 20 in.; depth, $6\frac{3}{4}$ in.; giving a total area of 966·5 sq. in. with lid and 705·5 sq. in. without. The distance of the nozzle of the atomizing gun from the sprayed surface was 12 in.–18 in., and the air pressure was 60–70 lb./sq. in. The rate of spraying was 6·8 c.c./min.

The boxes were kept beside a recording bimetallic thermograph and a recording hair hygrometer which were checked each week. In the later experiments the hair hygrometer broke down, so that only temperature records are available. The average temperature and the average relative humidity were calculated by measuring the average height of the curve for the period named.

Experiments 1 *and* 2

The first two experiments were made to determine approximately the amount of insecticide which should be used to form a lethal film, and to obtain some idea of the lasting properties of such a film.

The first box was sprayed with 53 c.c. of the insecticide and the second with 9·4 c.c. The first box was stained with the heavy coating; the amount applied to the second was about as much as is likely to be applied by a single medium spraying in practice.

The insects used in these two experiments were collected from the warehouse; 225 larvae of *P. interpunctella* and 96 larvae of *E. elutella* were put into an unsprayed box as controls. After 6 weeks 150 of the *Plodia* larvae and 66 *Ephestia* larvae were removed and used in the experiments; at the time of removal they were perfectly healthy. The remaining controls all emerged as moths.

The results are set out in Tables I and II and show that, under the given conditions, a film of insecticide of about 0·0053 g./sq. in. is lethal to the fully grown larvae of the two species, and that a film of this weight can retain its toxicity when kept in diffused light, for periods of up to 20 days.

These experiments were done at a time when the larvae are normally hibernating and many of them had to be removed from their cocoons. They were thus more susceptible than larvae under their normal conditions of migration.

A series of experiments based on the foregoing results was made during the summer months to obtain further information on the action of the film. The first experiment of this series was made to determine whether the oil alone could be lethal and the results are given in Table III.

The figures show that very little of the toxicity of the film can be attributed to the oil, because even when the oil was used at a dosage of about 0·029 g./sq. in. it had very little toxicity. It is noteworthy that in one of the series in which the insecticide was used for comparison with the oil film there was 15% survival. The insecticide used in this instance was the commercial extract. The two experiments with the insecticide which gave 100% kill were made with the extract standardized in the laboratory by the Seil acid method.

In the foregoing experiments the larvae were exposed to the action of the film for 7 days in order to eliminate as far as possible the factor of time of exposure, when assessing the properties of the film. In practice, it is desirable that the larvae should be killed after a shorter time of exposure and a series of experiments were made on new and 7-day-old films of approximately 0·0053 g./sq. in. of insecticide to determine how the length of exposure of the insect to the film affected the mortality. The results of this series are given in Table IV.

Table I

First preliminary experiment to test the effectiveness and lasting properties of films of approximately 0·8% pyrethrins I and II in white oil on the fully grown larvae of *P. interpunctella* and *E. elutella*: (*a*) Heavy film. The insecticide was applied to the interior of boxes of Canadian hemlock to produce a film of approximately 0·029 g./sq. in. of insecticide [53 c.c. of insecticide were used]. The larvae were exposed to the action of the film for 7 days. The film was kept in the dark. The season during which the larvae were exposed was from 17 January to 28 February. The total period of the experiment was from 17 January to 18 April.

Test no.	Age of film (days)	No. of larvae		Temperature range during exposure (° F.)			Relative humidity during exposure (%)			Max. period between exposure and death (days)	Temperature range during survival period (° F.)			Relative humidity during survival period (%)			No. surviving to pupate
		P. interpunctella	*E. elutella*	Av.	Max.	Min.	Av.	Max.	Min.		Av.	Max.	Min.	Av.	Max.	Min.	
1	0	150	50	45·5	48·0	44·0	77·4	86·0	72·0	50	45·0	51·0	38·0	74·7	94·0	51·5	0
2	7	200	—	44·8	48·0	41·0	76·8	94·0	66·0	60	45·2	55·5	38·0	72·0	94·0	45·0	0
3	14	200	—	44·0	48·0	40·0	76·0	90·0	53·0	60	45·7	55·5	38·0	71·0	90·0	45·0	1
4	21	100	—	42·0	48·0	38·0	70·0	86·0	51·0	53	45·8	55·5	38·0	70·6	90·0	45·0	0
5	28	100	—	48·0	53·0	46·0	76·4	90·0	58·0	50	46·5	55·5	38·0	69·7	90·0	45·0	0
6	42	100	—	44·0	46·0	42·0	76·0	80·0	65·0	49	46·6	55·5	38·0	66·4	88·0	45·0	0

Table II

Second preliminary experiment to test the effectiveness and lasting properties of films of approximately 0·8% pyrethrins I and II in white oil on the fully grown larvae of *P. interpunctella* and *E. elutella* Hb: (*b*) Light film. The insecticide was applied to the interior of boxes of Canadian hemlock to produce a film of approximately 0·0053 g./sq. in. of insecticide [9·4 c.c. of insecticide used]. The larvae were exposed to the action of the film for 7 days. The film was kept exposed to diffused light. The season during which the larvae were exposed to the film was from 7 March to 27 March. The total period of the experiment was from 7 March to 10 June.

Test no.	Age of film (days)	No. of larvae		Temperature range during exposure (° F.)			Relative humidity during exposure (%)			Max. period between exposure and death (days)	Temperature range during survival period (° F.)			Relative humidity during survival period (%)			No. surviving to pupate
		P. interpunctella	*E. elutella*	Av.	Max.	Min.	Av.	Max.	Min.		Av.	Max.	Min.	Av.	Max.	Min.	
1	0	100	50	40·0	46·0	38·0	61·6	71·0	45·0	61	47·3	60·0	38·0	67·7	88·0	45·0	0
2	7	100	—	46·0	52·0	42·0	72·8	87·0	60·0	54	49·4	60·0	40·0	68·7	88·0	51·5	0
3	20	100	—	49·0	52·0	44·0	63·8	73·0	54·0	81	52·7	62·0	40·0	66·5	88·0	38·0	0

Table III

Experiment to compare the effect of a film of the oil carrier alone with that of similar films containing 0·8% pyrethrins in Shell oil 24210. The films were made on the interior of boxes of Canadian hemlock and were kept in the dark. The test insects were migrating larvae of *P. interpunctella*. Period of exposure to the film was 7 days. The experiments were carried out between 25 June and 6 October 1937.

Insecticide	No. of larvae	Approximate amount of material per sq. in. (g.)	Temperature range during exposure (° F.) Av.	Max.	Min.	No. surviving to pupate	Temperature range during experiment (° F.) Av.	Max.	Min.	% kill
Shell oil 24210	100	0·024	64·0	68·0	60·0	88	64·4	74·0	60·0	12
,,	100	0·024	66·0	68·0	62·0	85	66·6	74·0	62·0	15
,,	100	0·0053	64·0	68·0	60·0	92	64·4	74·0	60·0	8
,,	100	0·0053	66·0	68·0	62·0	97	66·6	74·0	62·0	3
0·8% pyrethrins* in Shell oil 24210	100	0·0053	64·0	68·0	60·0	15	64·4	74·0	60·0	85
,, †	100	0·0053	66·0	68·0	62·0	0	66·6	74·0	62·0	100
,, †	100	0·0053	66·0	68·0	62·0	0	66·6	74·0	62·0	100

* Non-standardized commercial extract.

† Extract standardized by the Seil acid method.

Table IV

Experiment to investigate the effect of varying times of exposure to films of 0·8% pyrethrins in Shell oil 24210 applied at the rate of approximately 0·0053 g./sq. in. to boxes of Canadian hemlock. The films were kept in the dark. The insecticide was made up from an extract standardized by the Seil acid method. The test insects were migrating larvae of *P. interpunctella*. The experiments were carried out between 29 September and 24 November.

No. of larvae used	Age of film	Period of exposure to film	Temperature range during exposure (° F.) Av.	Max.	Min.	No. surviving to spin cocoons	Temperature range during experiment (° F.) Av.	Max.	Min.	% kill
100	New	30–45 min.	58·0	—	—	31	54·1	63·0	44·0	69
100	New	7 days	60·0	63·0	58·0	0	52·2	63·0	40·0	100
50	—	Controls	60·0	63·0	58·0	48	52·2	63·0	40·0	4
50	7 days	3 hr.	58·0	—	—	16	50·6	58·0	40·0	68
50	7 days	24 hr.	57·5	58·0	57·0	9	50·6	58·0	40·0	82
50	7 days	7 days	56·0	58·0	54·0	3	52·0	58·0	40·0	94
50	7 days	Controls	56·0	58·0	54·0	50	50·6	58·0	40·0	0

Table IV does not contain sufficient data to decide the minimum period of exposure which will give a maximum toxic effect for the particular film, but indicates that an exposure of 24 hours to films up to 1 week old will give fair percentage kill. It suggests that new films would be effective in a few hours. Further evidence on these points was obtained in the laboratory experiments.

Finally a short series of experiments was made to test the lasting properties of the film during the summer months, in case these properties should differ very greatly from those of films prepared in the colder weather. The results of these are shown in Table V.

Table V

Experiment to test the lasting properties of films of 0·8% pyrethrins in Shell oil 24210 applied in the summer at the rate of approximately 0·0053 g./sq. in. to boxes of Canadian hemlock. The films were kept in the dark. The insecticide was made up from an extract standardized by the Seil acid method. The test insects used were the migrating larvae of *P. interpunctella*. Experiments carried out between 9 August and 8 September.

No. of larvae	Age of film	Period of exposure to film	Temperature range during exposure (° F.) Av.	Max.	Min.	No. surviving to pupate	Temperature range during experiment (° F.) Av.	Max.	Min.	% kill
100	12 days	4 days	70·6	73·0	68·0	0	67·0	74·0	62·0	100
50	19 days	7 days	65·3	68·0	62·0	0	66·0	70·0	62·0	100

Table V shows that the film remains fully effective in the summer for periods of at least 19 days. The results of this experiment taken in conjunction with those of the experiments previously described show that the film will remain effective over a considerable period when kept at the normal temperatures of the English climate, in the dark or in diffused light.

Laboratory experiments

(*a*) *Experiments on migrating larvae of* Plodia interpunctella *Hb.*

A short series of experiments was made in the laboratory under more completely controlled conditions than it was possible to achieve in the semi-field experiments described above.

Small squares of Canadian hemlock of either 4 or 4½ in. were cut out and weighed. Each square was then sprayed in a spraying tower, which had been designed for testing contact insecticides. They were then re-weighed, the difference in weight giving the amount of spray deposited. The tower has been tested to make certain that the spray is evenly deposited over the square.

The squares prepared in this way were either put together to form a box in which the test insects were placed, or they were used singly. In the latter case the insects were put on the film and prevented from wandering off by means of a down-turned petri dish or filter funnel.

The standard adopted for the film was 0·0065 g./sq. in. of insecticide which was slightly higher than that in the semi-field experiments, since at least two applications should be made before moth emergence com-

mences. This amount corresponds to one heavy spraying. Owing to the difficulties of experimental technique, the standard could only be approximated. The actual concentrations are given for each experiment and the variations do not seem to be significant.

The first series of experiments were designed to amplify the data obtained in the semi-field trials on the minimum length of exposure of the insects to new films, necessary to obtain a maximum toxic effect. The results are given in Table VI.

Table VI

Experiment to determine the length of exposure of migrating larvae of *P. interpunctella* to new films of 0·8% pyrethrins in Shell oil 24210 on squares of Canadian hemlock necessary to kill. Three squares were used, the larvae being distributed over the three. Concentration of insecticide: Square 1, 0·0064 g./sq. in.; Square 2, 0·0072 g./sq. in.; Square 3, 0·0077 g./sq. in. Temperature during experiment: 68–72° F. Insects subsequently kept in an incubator at 73–75° F. and 70% R.H.

Time larvae exposed to film (min.)	No. used	No. surviving	% kill	Approximate survival period (days)
15	25	8	68	27
30	25	1	96	27
60	25	0	100	27
120	25	0	100	27
240	25	0	100	27
360	25	0	100	27
Controls	25	19	24	27

Table VI shows that an exposure period of 1 hour to a fresh film was completely effective under the conditions of the experiment.

It was obvious from the previous experiments that the activity of the film diminished rapidly with age, and a series of experiments was therefore made to test the minimum effective length of exposure to films 1 week old. The results of this series are given in Table VII.

Table VII

Experiments to determine the length of exposure of migrating larvae of *P. interpunctella* to 7-day-old films of 0·8% pyrethrins in Shell oil 24210 on squares of Canadian hemlock made up into a box, necessary to kill. Concentration of insecticide, 0·0062–0·0077 g./sq. in. Temperature during experiment, 68–72° F. Insects subsequently kept in an incubator at 73–75° F. and 70% R.H.

Time larvae exposed to film	No. used	No. surviving	% kill	Approximate survival period (days)
15 min.	25	21	16	27
30 min.	25	19	24	27
60 min.	25	18	28	27
120 min.	25	17	32	27
240 min.	25	17	32	27
360 min.	25	18	28	27
24 hr.	25	0	100	27
48 hr.	25	0	100	27
72 hr.	25	1	96	27
Control	25	25	0	—

Table VII confirms that the activity of the film has greatly decreased on keeping 7 days. A 6 hr. exposure only gives a very small kill. An exposure period lying between 6–24 hr. is necessary to secure a kill approximating to 100%.

(*b*) *Summary of the semi-field and laboratory experiments on the action of the film on migrating larvae*

A film of 0·005–0·007 g./sq. in. of insecticide on an absorbent wood, Canadian hemlock, will give either a complete or an almost complete kill of the larvae of *P. interpunctella* and, therefore, also of the more susceptible larvae of *E. elutella*.

The film will remain effective for at least a week and retains a high degree of toxicity for at least 19 days at temperatures commonly prevailing in England. The period of exposure of the insects to the film necessary to obtain a maximum effect varies with the age of the film. An exposure of 1 hour to freshly prepared films is enough to secure a high percentage kill, but a period of between 6 and 24 hours is necessary for week-old films. The pyrethrum is the main toxic agent, the oil in the insecticide having little or no effect.

These data apply to films that are in the dark or in diffused daylight.

(*c*) *Laboratory experiments on the moths of* Plodia interpunctella

It has been stated, previously, that practical control is obtained by the action of the film on the moths, the action on the larvae being of secondary importance. When, therefore, the above information on the activity and lasting properties of the film had been obtained by experiments with the larvae, it was necessary to get definite information on the susceptibility of the adults. Once a comparison had been made between the susceptibility of the larval and the adult instars, the results of the experiments made on the larvae could be applied to the adults. A series of experiments was, therefore, carried out in the laboratory to test the susceptibility of the moths of *P. interpunctella* to films of insecticide. Preliminary experiments showed no appreciable difference in the susceptibility of moths of *E. elutella* and *P. interpunctella*, so the latter were used, because they were more readily available.

The experiments were made whenever moths were available, because of the difficulty of obtaining large numbers of known age at any given time. In spite of this lack of standardization of the biological material, the experiments should afford a useful index of the effect of the films on

the moths; it is noteworthy that none of the 375 moths exposed to the films survived.

When moths are placed on new or old films they are rapidly paralysed. The most resistant moths were paralysed in 30 min. on an old film; 15 min. is usually sufficient on fresher films. The moths remain in a state of paralysis until they die, although signs of life consisting only of an ability to twitch the legs and antennae may persist for several days or as long as the normal adult life. Controls were kept during each experiment to ascertain that no deaths would normally occur during the experiment. The treated moths were kept to see whether any would recover, but none did. The method of making the films was similar to that described above for the experiments on the larvae. The results are given in Table VIII.

Table VIII

Results of experiments on the effect of films of 0·8% pyrethrins I and II in white oil applied to wood of Canadian hemlock on moths of *P. interpunctella*. Experiments made in the laboratory at temperatures between 60 and 70° F.

Age of film	Amount of insecticide in g./sq. in.	No. of insects used	Age of insects	Period before insects moribund (min.)	Period insects exposed to film (min.)	% kill
30 min.	0·0062–0·0072	25	Unknown	8	15	100
Controls	—	25	,,	—	—	0
6 hr.	0·0064–0·0077	50	,,	10	10	100
24 hr.	0·0058–0·0070	16	,,	6	6	100
Controls	—	13	,,	—	—	0
8 days	0·0062–0·0072	50	,,	10	10	100
Controls	—	25	,,	—	—	0
8 days	0·0064–0·0075	37	1–3 days	14·5	15	100
Controls	—	15	,,	—	—	0
14 days	0·0058–0·0070	16	Unknown	24	33	100
Controls	—	8	,,	—	—	0
17 days	0·0062–0·0072	33	1–6 days	13·5	15	100
Controls	—	10	,,	—	—	0
19 days	0·0058–0·0070	16	Unknown	—	30	100
Controls	—	4	,,	—	—	0
26 days	0·0058–0·0070	14	,,	14	30	100
Controls	—	8	,,	—	—	0

(*d*) *Summary of the experiments on moths*

These experiments indicate that a film of the insecticide within a range of 0·0058–0·0077 g./sq. in. on an absorbent wood, such as Canadian hemlock, will remain effective against these moths for at least 26 days, if protected from dust and strong sunlight.

A comparison of the results in Table VIII with those of Table I–VII shows that the moths of this species are very much more susceptible than the full grown migrating larvae.

The success of the process in practice is due to the great susceptibility of the moths, against which stage in the life-history the method is directed.

Preliminary experiments on the effect of pyrethrum-white oil films on miscellaneous insects

After it had been found that pyrethrum-white oil films were highly toxic to the adults and larvae of *P. interpunctella* and *E. elutella*, a few preliminary experiments were made with these films on other species of indoor insects.

The experiments were done in the laboratory and the technique was similar to that used for *P. interpunctella.* Small squares of Canadian hemlock were coated with the insecticide and the material was allowed to soak into the wood for a period of not less than half an hour. The insects were placed on the film after the first half hour and before the lapse of 24 hr.

In the first series of experiments the squares were made up into a box and the insects were placed inside it; in the later series single squares were used and the insects were confined to the square by means of an upturned petri dish or filter funnel. The insects were not standardized in any way, but were of varying ages and had been bred under a variety of conditions. The experiments can, therefore, only be regarded as a guide to the relative susceptibility of the various species and the figures for percentage kill as rough approximations. The results of these experiments are given in Tables IX*a* and IX*b*.

Table IX*a*

Preliminary experiments on the effect on miscellaneous insects of films of 0·8% pyrethrins in white oil on Canadian hemlock. Films not more than 24 hr. old. Temperature range during the experiments, 60–66° F.

Species	Instar	Amount of insecticide in g./sq. in.	Time of exposure	No. of insects used	No. surviving	% kill
Lasioderma serricorne	Adults	0·0064–0·0076	15 min.	27	2	92·6
F.	,,	—	Controls	25	20	20
Sitodrepa panicea R.	,,	0·0062–0·0072	30 min.	26	0	100
	,,	—	Controls	25	22	12
Tineola bisselliella	,,	0·0062–0·0072	15 min.	16	0	100
Hüm	,,	—	Controls	10	9	10
	Larvae	0·0062–0·0072	30 min.	25	0	100
	,,	—	Controls	25	23	8
Tribolium castaneum	Adults	0·0064–0·0076	15 min.	23	0	100
Hort.	,,	—	Controls	25	24	4
Trogoderma versicolor	,,	0·0062–0·0072	30 min.	26	1	96·2
Creutz	,,	—	Controls	25	22	12
	Larvae	0·0062–0·0072	30 min.	25	24	96
	,,	—	Controls	25	25	0
Oryzaephilus	Adults	0·0068	30 min.	25	0	100
surinamensis L.	,,	—	Controls	25	22	12

Table IX *b*

Preliminary experiments on the effect on *Cimex lectularius* L., *Calandra granaria* L. and *C. oryzae* L. of films of 0·8 % pyrethrins in white oil on Canadian hemlock. Films not more than 24 hr. old. Temperature range during the experiments, 60–66° F.

Species	Instar	Amount of insecticide in g./sq. in.	Time of exposure	No. of insects used	No. surviving	% kill
Cimex lectularius L.	Adults	0·0062–0·0072	30–45 min.	24	1	95·8
	,,	—	Controls	25	25	0
	,,	0·0064–0·0076	60 min.	23	5	78·3
	,,	—	Controls	25	25	0
	,,	0·0065	24 hr.	52	0	100
	,,	—	Controls	51	51	0
	Nymphs	0·0065	24 hr.	53	0	100
	,,	—	Controls	50	50	0
	Adults	0·0131	24 hr.	49	0	100
	,,	—	Controls	52	51	1·9
	Nymphs	0·0131	24 hr.	50	0	100
	,,	—	Controls	50	50	0
Calendra granaria L.	Adults	0·0065	2 hr.	21	7	66·7
	,,	—	Controls	24	16	33·3
	,,	0·0067	24 hr.	53	0	100
	,,	—	Controls	50	49	2
	,,	0·0129–0·0132	24 hr.	77	0	100
	,,	—	Controls	71	59	16·9
Calandra oryzae L.	,,	0·0065	24 hr.	52	0	100
	,,	—	Controls	50	49	2

Tables IX*a* and IX*b* show that the adult stage of a number of indoor pests is very susceptible to the action of the film, but *Calandra granaria*, *C. orzyae* and *Cimex lectularius* are important exceptions and the larvae of *Trogoderma versicolor* are also very resistant.

Some additional experiments were carried out on the adults of the two species of *Calandra* and the adults and nymphal stages of *Cimex lectularius* because of the economic importance of these insects. These results indicate that a complete kill of these species may be obtained with a long exposure, but it must be remembered that only small numbers of insects are used so that the results are only indicative. The experiments suggest that the film may be useful against a variety of industrial insects. It might be of particular value in cleaning up the residual infestation in empty warehouses and other buildings where fumigation is difficult or undesirable. However, before concrete recommendations can be made, work must be done on the susceptibility of the various species. Preliminary experiments indicate that a film containing an organic thiocyanate may be more toxic to *Cimex lectularius* than a pyrethrum film, so that by the choice of a suitable insecticidal agent, the method might be useful against all species of indoor pests.

Field scale experiments on E. elutella and P. interpunctella

The results of the preliminary experiments showed that if the boxes and exposed surfaces in a warehouse were sprayed directly by means of atomizing guns, a film of insecticide could be formed which would be lethal to insects settling upon it and would act as a protection to the goods inside the boxes against infestation from without. The film would also prevent infestation spreading outwards from inside the box.

One warehouse in Manchester of a capacity of 268,000 cu. ft. was available for the experiment. It stored approximately 650 tons of dried fruit annually. This warehouse was infested with *E. elutella* and *P. interpunctella,* chiefly the former. The object of the experiment was to prevent the moths emerging from hibernating larvae in the premises from reaching and infesting the stored dried fruits. The fruit had all been fumigated on arrival and was substantially if not completely free from infestation.

The method adopted was to start applying the film about the middle of May, approximately a fortnight before the first moths would emerge and to apply it twice a week during the emergence period of the moths.

The apparatus which was used is of the type already described (Potter, 1935) for use in the mist method. For the present method a small air compressor is sufficient, because it is not necessary to put a large quantity of material into the warehouse in a short space of time, as in the mist method. The air compressor was driven by a one H.P. motor and delivered about 6 cu. ft./min. of free air. This machine cost about £40 and works one gun. In this instance an H.M. oil-spray gun of Aerograph Co. was employed. The air pressure was about 45 lb./sq. in.

No definite rule can be laid down for adjusting the degree of atomization, because it will depend on the accessibility of the area to be coated; in general, as coarse an atomization as possible consistent with even coating and lack of staining is desirable.

(a) *Experimental procedure*

Spraying was carried out twice a week from 15 May to 30 September 1936. From 15 May to 16 June a strength of about 0·65% pyrethrins in white oil was used, but from then onwards the strength was raised to 0·8% pyrethrins. An average quantity of 9200 c.c. of spray was used for each spraying of the warehouse of 268,000 cu. ft. and its contents; the contents varied in amount.

(b) *Assessment of results*

The results may be assessed in two ways. First, by inspection of the fruit for infestation, together with an inspection of the premises for the bodies of the moths which would have attacked the fruit; secondly, by a comparison of the infestation occurring in the year in which the process was used, with that of the previous year when no control measures had been adopted in the warehouse. Both these assessments were made.

The bodies of the moths which had been killed by the film, when they could be found, were collected and examined to determine the identity of the moths and their sex. The results of this work are given in Table X.

Table X

Results of examination of the bodies of moths collected from the Manchester warehouse during the 1936 season.

Ephestia elutella		*Plodia interpunctella*		*Borkhausenia pseudospretella* St.		*Ephestia cautella* Wlk.		Unidentified	Total	Total wings not expanded
♂	♀	♂	♀	♂	♀	♂	♀			
96	88	2	4	1	2	0	1	28	222	130

The numbers recorded in the above table are not large, but they represent only a small proportion of the moths which were present, since the majority were in inaccessible places and could not be examined. Experience has shown that, if numbers such as these can be found, there are sufficient moths actually present to set up a very serious infestation.

(c) *Summary of results of the field scale experiments*

An interesting point brought out by Table X is the high proportion of moths which were killed before they were able to expand their wings. The following explanation may account for this: as soon as the moths emerge from the pupae they crawl on to an exposed surface to expand their wings; this brings them into contact with the insecticidal film and a considerable proportion of them are disabled and killed before they have expanded their wings. This fact also suggests very strongly that the film formation is the effective part of the process and that the moths have not been killed by direct action of the spray.

Three careful inspections were made of the stored fruit in 1936, on 8 June, on 15 July and on 3 September. Another inspection was made on 14 March 1937. These were in addition to those made by the warehousemen during the normal operations of moving the piles and sampling the cases of fruit. Throughout the season no infestation was found.

As evidence of the difference between the state of the fruit when film spraying had been adopted and when no protective measures had been taken, as in 1935, it can be quoted that inspections in 1935 showed that every pile of fruit was infested to a greater or lesser extent. It was necessary to refumigate 56·375 tons during the 1935 season out of a total of 661·885 tons. This suggests that the film spraying was effective, since there was as much chance of infestation in 1936, when none occurred, as in 1935.

General summary and conclusions

1. A series of experiments and a field scale trial have been made of a film spraying method for the protection of stored goods from infestation by *E. elutella* and *P. interpunctella*.

2. It is shown that where the goods have been efficiently sterilized before storing, reinfestation by these two species in the warehouse can be prevented completely, as far as could be ascertained.

The procedure is to spray the interior of the warehouse and the cases stored in it with an insecticide consisting of a solution of 0·8% pyrethrins in a white oil, the constitution of which is given. The apparatus and method of spraying are described. Spraying should be started some time before the moths are expected to emerge; in England about 15 May, and should be continued twice a week throughout the period of moth emergence, which may continue until 30 September.

3. The laboratory and semi-field scale experiments, together with data obtained during the commercial working of the process, show that control is obtained by a protective film of insecticide, which is formed and maintained throughout the period of moth emergence.

A film of about 0·006–0·007 g. of insecticide per sq. in., which is about the amount applied in practice in a single heavy spraying or two light sprayings, on an absorbent wood such as Canadian hemlock, will remain effective against moths of the two species for at least 26 days. It is highly effective against the migrating larvae for a week or longer.

The moths were paralysed and ultimately killed by an exposure to the insecticide of not more than 30 min.; the larvae need to be exposed to the action of the film for periods of 1–24 hr., depending on the age of the film.

4. It was shown that the oil plays very little part in the toxic effect of the film.

5. A short series of experiments indicate that the method of using a protective film of insecticide might be usefully applied in the control of

other indoor insects. Probably its most useful application is in cleaning up the residual infestation in empty warehouses and other buildings, in cases where fumigation is not practicable or desirable.

6. The effectiveness of this method of prevention of infestation by *E. elutella* and *P. interpunctella* has been proved by its adoption for the protection of 30,000 tons of dried fruits in warehouses in London, Liverpool, Manchester and Glasgow, with complete success.

7. The method has also been adopted for the protection of cacao and tobacco in London and elsewhere. In these instances, although a great reduction of infestation was observed, it was not possible to assess the results accurately, because the goods were not sterilized before being brought into store and therefore fresh infestation was continuously brought into the warehouse.

8. The advantages of the method are that it can be used without interference with the normal routine of storage and involves no extra handling of the goods.

It is also a continuous protection from infestation during periods when this is likely to occur. This is a very important point when goods are stored in places where other infested goods may also be stored.

This method has the further advantage, that it will localize and check the spread of infestation if this is brought into a building in which the method is used.

Finally, compared with fumigation, it is cheap and is devoid of risk to human beings.

9. Although the insecticide described in this paper has been successful, it is not necessarily the only possible one for use in this method. Preliminary trials have shown that one of the organic thiocyanates, β butoxy β' thiocyanodiethylether has a marked toxicity when applied in this way and may compare favourably with pyrethrum for some purposes.

I am greatly indebted to Mr J. J. Scouler, Secretary of the Australian Dried Fruits Board, for giving me every facility in the warehouses storing the Board's dried fruits, and to Messrs A. E. Green and C. R. Rainbird, warehouse representatives of the Board, for their assistance. My thanks are also due to Mr Tomlinson, Manager of the Manchester warehouse, for his willing cooperation in the conduct of the field experiment.

The standardization of the pyrethrum extract was carried out in Dr Tattersfield's laboratory at the Rothamsted Experimental Station, and I am indebted to Dr Tattersfield for his courtesy and to Dr Martin for his help in the work of the estimation.

The work was carried out from the Entomology department of the Royal College of Science, under the direction of Prof. J. W. Munro.

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INVESTIGATIONS UPON THE CONTROL OF OAT SICKNESS BY THE ADDITION OF CERTAIN CHEMICAL SUBSTANCES TO SOIL INFECTED WITH *HETERODERA SCHACHTII* SCHMIDT

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(With Plate XXXVI)

CONTENTS

1. Introduction

In 1935 a paper on the nematode *Heterodera schachtii* with special reference to the oat race in Britain was published in the *Journal of Helminthology* (Edwards, 1935). The general field observations made of infested areas in Shropshire during 1933 and 1934 were discussed, as well as the results of experiments designed to ascertain the range of host plants liable to attack by this strain. It was shown that great practical difficulties would be involved in planning suitable crop rotations whereby the oat race could be controlled, since the parasite is capable of attacking and reproducing freely not only on cereals but also on rye-grasses and red clover. In view of these circumstances, the problem of affording direct protection to cereal crops against invasion of the nematode by the addition of chemical substances to affected soil was next investigated in 1936 and 1937. The present communication records the results obtained during this subsequent study.

2. Method

The method adopted to determine the protective value of the different materials was to grow oats in earthenware flower pots, 11 in. in diameter, which were filled to within 1 in. of the top with soil previously treated with the respective dressings. The soil used, the same in all pots, was heavily infested with the cereal race of *H. schachtii*, and had been taken from a field that was worthless for the growing of cereals on account of "oat-sickness". The field was known to have produced until recent years excellent yields of grain and was still in a satisfactory state of fertility, judging by the growth of other crops and by the chemical analysis of the soil. The latter was as follows:

	%
Organic matter	4·11
Total nitrogen	0·20
Available phosphoric acid (P_2O_5)	0·021
Exchangeable potash (K_2O) ...	0·016
Carbonates (as $CaCO_3$)	0·210
Lime requirement ($CaCO_3$) ...	Nil
*p*H	7·1

These data show that there was no deficiency in essential "plant food", which might account for the failure of the cereal crops.

When the soil was air-dried it was lightly pulverized and all the coarser material removed with a ½ in. sieve. The soil was then thoroughly mixed until it became uniform in general appearance and cyst concentration. When the cyst counts of several consecutive samples of identical weight taken at random from the heap of soil were compatible with one another, it was assumed that homogeneity with regard to cyst distribution had been attained.

Special precautions were taken to obtain a thorough incorporation of each experimental substance with the entire soil of its respective pot. The contents of each pot selected for treatment were turned out on to a clean sheet of strong brown paper on a table and the chemicals incorporated with the soil by hand. The resulting small heap was then passed five times through a sieve of ⅛ in. mesh, care being exercised that no two consecutive trowelfuls came from the same part of the heap. All signs of "stratification" due to unevenness of distribution had disappeared after the third sifting. After this intimate admixture with the chemical, the soil was returned to the pot and kept moderately moist until the sowing of the oat seed.

The tests were carried out in triplicate, a series of three pots being allotted to each treatment, together with a similar series left untreated. An application of artificial manure at the rate of 6 cwt. to the acre was given to all the pots, including the controls, a few days before sowing. It was composed of two parts of sulphate of ammonia, three parts of superphosphate and two parts of sulphate of potash. All the pots were sown, with the variety "Victory" in the first week in April, at the rate of 30 seeds per pot at a depth of ½ in. They were then plunged in groups, according to their series, in a bed of ashes and soil in an open air enclosure with a south-westerly aspect, being carefully watered throughout the season in such a way as to approach as closely as possible field conditions.

At intervals during the course of the experiments, quantitative observations were made on the plants in all series to obtain an analysis of the effects of different treatments upon (a) germination, (b) vegetative growth, (c) panicle production and yield of

grain, and (*d*) degree of infestation of the roots by the nematode. Of these four factors vegetative growth (*b*) proved the least satisfactory, since it was not found possible to assign to it any definite standard of measurement apart from that given by the number of plants and average height of shoots at two distinct periods during their early development.

3. Treatments tested

The following substances were intimately mixed with the soil of the respective series of pots:

1. Ferrous sulphate at the rate of 15 cwt. per acre.
2. Precipitated ferric oxide at the rate of 6 cwt. per acre.
3. Natural ferric oxide at the rate of 6 cwt. per acre.
4. Ferric chloride at the rate of 12½ cwt. per acre.
5. Nitrate of soda at the rate of 60 cwt. per acre.

6–10. Calcium cyanamide at rates of 20, 40, 60, 80 and 100 cwt. per acre, respectively.

The ferrous sulphate, ferric chloride and the different dressings of calcium cyanamide were added to the soil 25 days before the seed was sown, and the ferric oxides as well as the nitrate of soda at the time of sowing.

The various compounds of iron were included in the investigation in view of the promising results given by them in experiments conducted by Hurst & Triffitt (1935) on control of the potato sickness associated with *H. schachtii*. The nitrate of soda series was prepared with the object not only of observing whether this nitrogenous fertilizer would in any way assist the plants to overcome the adverse effects of oat sickness, but also for purposes of comparison with the calcium cyanamide series. Since the addition of the calcium cyanamide would considerably increase the nitrogen content of the soil, any beneficial results obtained by its use might be due to the abundance of available nitrogen.

4. Discussion of data obtained in 1936

(1) *Effect of treatments on vegetative growth*

It was possible throughout the growing season to detect marked differences in the growth and vigour of the oat plants under the various treatments. The plants in the untreated soil produced normal growth for the first few weeks, but this was followed by a disastrous check in the growth rate accompanied by the customary manifestations of the oat-sickness associated with root invasion by *Heterodera schachtii*.

For some weeks after germination, the plants in the series of pots

treated with calcium cyanamide in amounts exceeding 40 cwt. to the acre showed definite indications of distress, being fewer in number (Table I series 4, 5, 6), somewhat irregular in size, and exhibiting a definitely slower rate of growth than the controls. These adverse effects were in direct relation to the amount of the chemical added to the soil, being most pronounced in the pots which had been given the heaviest application. This is well exemplified by the average height of the shoots recorded for the plants under the different treatments some seven weeks after sowing as shown in the sixth column of Table I. It will be seen that the shoot growth in the series (3, 4, 5, 6) which had received dressings of cyanamide corresponding to 40, 60, 80 and 100 cwt. per acre measured on an average 12, 25, 45 and 56 mm. less in height respectively, than that of the plants in the untreated soil. From this time onwards, growth in the pots treated with these heavier dressings of cyanamide gradually accelerated, until by mid-July the plants possessed foliage conspicuously superior to that of the controls, both in regard to its amount and the depth of colour. The average height of the shoots in each series at this later date is given in the seventh column of Table I where it will be noted that the shoot growth for each of the four treatments averaged 190 mm. between 16 June and 10 July, as against 52 mm. for the controls during the same period, that is, the growth rate in height of the plants had been more than trebled by these soil dressings. This pronounced disparity was maintained until the plants had attained full maturity and were finally examined.

On the other hand, at the outset the plants in the ferrous sulphate (series 9), and particularly in the ferric chloride (series 10) pots developed more rapidly than those in any of the other treated ones or in the controls (series 1). As the season progressed, this stimulating influence on the rate of foliage growth became more and more apparent and by mid-June was most striking. It was calculated that these plants were at this date approximately 7 days more advanced in growth than those in the control pots; there was on 16 June a difference on an average of 48 and 75 mm. between the height of the plants in the controls and those in the ferrous sulphate and ferric chloride soils, respectively (see Table I, sixth column). After the second week in June, this difference gradually became less apparent and by mid-July had become reduced in both instances to about 25 mm. (Table I, seventh column). There could be no doubt from the general appearance of the foliage that the plants in both these treated series were, at this stage, seriously affected by oat sickness although marked symptoms had been absent heretofore.

Table I. *Effect of different treatments on (a) establishment, growth and yield of oats in 1936, and (b) infestation of the roots of oats by* H. schachtii *in 1936 and 1937*

Series	Treatment	Serial no. of pot	No. of plants established		Average height of shoots		No. of panicles	No. of fertile grain	Wt. of fertile grain	No. of cysts on outer roots	
			12 May	15 Sept.	16 June cm.	10 July cm.			g.	1936	1937
1	Untreated (control)	1*a*	26	13	12·5	17·5	7	19	0·52	35	142
		1*b*	24	10	12·6	17·7	7	21	0·56	38	153
		1*c*	25	12	12·8	18·1	8	23	0·63	43	149
2	Cyanamide 20 cwt.	2*a*	27	24	16·6	34·6	23	184	5·73	89	151
		2*b*	26	23	16·3	34·2	23	174	5·24	79	141
		2*c*	24	22	16·6	34·3	22	170	5·42	85	146
3	Cyanamide 40 cwt.	3*a*	21	20	11·2	30·0	20	252	8·39	22	91
		3*b*	24	22	11·4	30·4	19	233	7·74	28	105
		3*c*	23	23	11·6	30·5	22	241	8·06	26	102
4	Cyanamide 60 cwt.	4*a*	21	21	10·4	29·2	21	285	9·34	13	78
		4*b*	20	20	10·0	28·6	18	272	8·90	12	78
		4*c*	20	19	9·9	28·8	19	269	8·84	9	69
5	Cyanamide 80 cwt.	5*a*	17	17	8·0	27·4	17	285	9·39	0	48
		5*b*	19	18	8·5	27·4	18	283	9·45	0	49
		5*c*	18	17	7·8	26·9	16	271	8·95	0	44
6	Cyanamide 100 cwt.	6*a*	18	17	6·6	25·9	17	278	9·07	0	0
		6*b*	19	19	7·4	26·5	19	296	9·69	0	0
		6*c*	18	18	7·1	26·2	17	284	9·25	0	0
7	Precipitated ferric oxide	7*a*	24	14	13·7	17·9	8	47	1·23	52	141
		7*b*	25	16	13·8	18·5	11	45	1·24	56	149
		7*c*	26	14	14·1	18·1	7	38	1·20	46	150
8	Natural ferric oxide	8*a*	25	14	13·7	18·3	10	36	0·92	48	151
		8*b*	24	11	13·2	17·7	7	32	0·93	47	148
		8*c*	22	12	13·5	17·7	8	29	0·89	43	140
9	Ferrous sulphate	9*a*	21	12	17·5	20·3	9	59	1·79	55	147
		9*b*	23	16	17·6	20·5	12	71	1·98	64	152
		9*c*	24	14	17·2	20·0	10	57	1·86	56	143
10	Ferric chloride	10*a*	23	17	20·3	20·5	13	76	1·99	67	149
		10*b*	22	15	20·2	20·4	10	67	2·08	61	131
		10*c*	25	17	19·9	20·3	12	72	1·93	60	144
11	Sodium nitrate	11*a*	22	16	13·6	18·1	10	34	0·89	46	139
		11*b*	24	15	13·7	18·7	13	30	0·90	41	147
		11*c*	25	18	14·0	18·3	11	29	0·88	42	153

The plants in the series treated with nitrate of soda and with precipitated and natural ferric oxides (nos. 7, 8, 11), were essentially similar to one another in general appearance throughout the season, and showed but little improvement over the controls. In contrast, those growing in soil which had received calcium cyanamide at a rate equal to 20 cwt. per acre were, in comparison with the controls, at all times characterized by their excellent growth rate and, as in the case of the other cyanamide treated series, by the dark green colour of their foliage as well as an entire absence of symptoms suggesting the presence of oat sickness.

(2) *Effect of treatments on yield of crop*

The produce was harvested from each pot on 15 September, and the numbers of plants and panicles recorded. When the oat grains were being threshed out some weeks later, the total yield from each pot was weighed out separately. The tabulated results are set out in Table I, where it will be seen that the degree of oat sickness was of a severe and fairly uniform order throughout the soil used in the present experiments, judging by the yields of grain obtained in the different control pots. From the 30 seeds sown in each of the three untreated pots the number of plants produced out of the possible numbers were, respectively, by 12 May, 87, 80 and 83 % and at harvest time 43, 33 and 40 %. Between these dates the control pots lost on an average 44 % of their plants, no doubt mainly due to the effects of oat-sickness. Further serious features associated with the disease are demonstrated in the control pots by the poor panicle production per unit area, and by the low weight of grain produced per panicle. Of the plants at harvest time in this untreated series, approximately a third failed to produce panicles, even though they were fewer in numbers at that date per unit area, and consequently, had more space for better shoot development than those in the cyanamide series.

The figures in the final columns of Table I indicate that all the soil treatments tested had some effect upon the crop, though in some instances, the differences are probably not significant. The greatest increase in weight of grain per unit area was found in those pots which received calcium cyanamide in dressings exceeding 40 cwt. to the acre, being on an average for these three series (4, 5, 6) 9·21 g. per pot compared with 0·57 g. per pot for the untreated soil. The same series also gave the highest number of fertile grains per panicle, averaging 15·8 as against 2·9 per ear for the controls, and 9·7 in the two series (2, 3) treated with cyanamide at the lower rates. It is difficult, however, to say how far the larger numbers, and therefore, more crowded condition of the

plants in the less heavily dressed series (2, 3) might have influenced the final results. The average numbers of plants per pot at harvest time were 18·4 for series 4, 5 and 6, and 22·3 for series 2 and 3. The significant differences in the yields of crop in the various cyanamide-treated series might therefore be due, at least to a certain extent, to the factor of competition, and not entirely to the degree of protection afforded to the plants from the deleterious effects of oat-sickness.

The data in Table I, concerning the relative value of the substances applied, other than calcium cyanamide, indicate that the effects upon panicle production and yield of grain, at the concentration tested, are doubtfully significant. As in the control series, seed of small size and poor quality was produced. Calcium cyanamide, on the other hand, favoured the development of the ear so that not only did it produce crops with high numbers of grains per panicle, but also produced the largest grains under the conditions of the experiments.

It would seem that the complete failure of the various dressings, other than calcium cyanamide, to afford appreciable protection of the plants from the pathological conditions associated with oat sickness, in the circumstances described when each was intimately mixed with the soil and given the desired degree of moisture, holds out little hope of ultimate success in combating the disease by means of any of these treatments when working on a field scale.

(3) *Effect of treatments on infestation by the nematode*

The technique adopted in the present investigation to determine the effects of the various soil treatments on the degree of infestation of the plants by the nematode was to count the number of encysted females actually protruding from the exterior of the roots (Triffitt, 1929). For the purpose, the contents of each pot were carefully turned out in an intact mass on 5 August and examined. At this date the major proportion of the root system was exposed upon the surface of the mould of soil and the white-stage females were clearly visible. The cyst counts carried out in this manner on the oat plants are set out in Table I, column 11, where it will be noted that none of the substances tested, with the exception of calcium cyanamide, had reduced the amount of eelworm infestation as indicated by the number of cysts on the roots. The most successful results are seen to have followed the application of calcium cyanamide in dressings at the rate of not less than 80 cwt. to the acre when production of cysts was completely suppressed (series 5, 6). A high degree of efficiency, as indicated by freedom from cysts on the roots, was

also obtained by the use of cyanamide at 60 cwt. per acre (series 4), the cyst population being only 28·2 % of that of the plants in the untreated series. Pl. XXXVI, Fig. 1 shows the marked difference between the root systems of typical plants from series 4 and from the control series. The inhibitory influence of cyanamide on cyst formation was decidedly less marked where it was used at the rate of 40 cwt. per acre, the infestation having only been decreased by about a third (series 3).

In contrast to the heavier dressings of calcium cyanamide tested, an application corresponding to 20 cwt. to the acre actually increased the nematode infection, the number of cysts on the roots being rather more than double the average control count. Slight increases in the eelworm infestation of the roots also followed all the other treatments included in the experiments. The intensity of the infection was, in general, directly in proportion to the vigour of the host plants. Thus, the series which showed the most luxuriant growth and the highest yield of grain also produced not only the best root development, but supported the largest eelworm population. The experiments were not sufficiently comprehensive to indicate why the oats grown, for instance, in the soil treated with calcium cyanamide at the rate of 20 cwt. to the acre were able to support with relatively little harm a cyst concentration twice as great as that which proved fatal to the plants in the untreated soil. Essentially similar trials carried out by the writer (1937) in 1936 on a field scale, against the potato race of the nematode gave results of the same kind. It was then discovered that the addition of these chemicals to the soil had delayed successfully eelworm invasion during the early growth of the plants, as shown by the time required for the protrusion of the female cysts from the roots, and that a marked correlation existed between the intensity of the sickness and the degree of the inhibitory action produced in this direction by the different treatments. It would seem that, as with potatoes (Carroll & McMahon, 1935), where the onset of the nematode attack is retarded until a good root system has been established, the effect on further growth and yield of the plants is comparatively small and under such circumstances the plants exhibit a high degree of tolerance of *H. schachtii*.

5. Discussion of data obtained in 1937

After harvesting in 1936, the plants in all pots were cut off at ground level, and the roots left undisturbed in the soil until a few days before re-sowing with oats in 1937. The contents of each pot were then turned out, broken up (neither roots nor cysts being removed) and thoroughly

mixed before returning to the pot. Oats were again sown, no treatment other than a dressing of mixed organic fertilizer at the rate of 6 cwt. to the acre being given. The latter consisted of 2 parts sulphate of ammonia, 4 parts superphosphate and 1½ parts sulphate of potash. The same variety ("Victory") was used and the sowing date, 15 April, was the same as in the previous year.

(1) *Effect of treatments on growth and yield*

Growth started quite normally in all pots, irrespective of the chemical treatment given to the soil in 1936. By the end of June considerable difference could be seen, however, between the plants in the series treated with calcium cyanamide in amounts exceeding 40 cwt. to the acre and those in the other series. The former appeared normal, no marked distinction being observed between those in soil treated at the rate of 60 cwt. per acre and those in which heavier dressings of the substance had been used. The other series, including the controls, were closely similar to one another, the plants exhibiting the usual symptoms of heavy parasitization by *H. schachtii*. An exception must be noted in the case of the pots which received calcium cyanamide at the rate of 40 cwt. to the acre. The plants in these pots, though somewhat stunted in growth, did not show symptoms of the disease so noticeably as the controls.

Inspections later in the season confirmed earlier impressions. The plants in the series receiving not less than 60 cwt. of calcium cyanamide per acre remained free from any signs of attack and grew normally with the production of good panicles. The plants of all the other treated series, on the other hand, remained typically diseased and showed at no time any improvement over the controls, except perhaps those grown in soil treated with calcium cyanamide in an amount equivalent to 40 cwt. per acre, but even the best of these could not be considered normal. Unfortunately, the ripening grain was attacked by birds and it was not found possible to obtain reliable numerical data beyond the panicle emergence stage.

(2) *Effect of treatments on infestation by the nematode*

The same technique as in 1936 was adopted to ascertain the influence of the treatments on infestation of the plants by the nematode. The contents of each pot were turned out on 3 August; the detailed figures of cyst counts for all the treatments being presented in the final column of Table I. It will be seen that only the plants grown in soil treated with calcium cyanamide at the rate of 100 cwt. to the acre remained free of

eelworm infection, as indicated by the absence of white stage females on the roots. Unlike the results obtained in 1936, the plants in the series dressed with 80 cwt. per acre of cyanamide bore definite evidence of attack but, on the average, the number of cysts present was only about a third of the count on the controls. Although the application at the rate of 60 cwt. per acre had limited the nematode infestation in 1936 to a negligible amount, and had enabled the production of a vigorous, healthy crop for two seasons after treatment, nevertheless it failed to prevent the eelworm population from increasing by the end of this period to a total nearly twice its original figure, i.e. that found on the control plants in 1936. This dressing had, however, produced appreciable relief even to the plants grown in the second year after treatment, from invasion of the root tissue by the nematode, as indicated by a comparison of the cyst counts recorded for this series in 1937 with those for the control in the same year. It would seem that the beneficial influence which also followed the use of 40 cwt. of calcium cyanamide to the acre, was apparent in the second year also but to a much less extent than in the case of the heavier applications of this substance. None of the other treatments applied in 1936 had been of any value in preventing heavy parasitization of the oats grown in 1937 since, in no instance, did the number of cysts on the roots differ materially from the average control count.

It will be seen from Table I that, as a result of growing oats for two years in succession, a great increase in the number of cysts in the roots exposed on the surface of the mould of soil in the pots occurred, except where the heavier dressings of calcium cyanamide had been administered. In the control series the infection approximately quadrupled; the cyst population in the pots which received 20 cwt. of calcium cyanamide per acre had doubled, that in the sodium nitrate and ferric oxides series had trebled, and that in both ferrous sulphate and ferric chloride series had increased about two and a half times. It must not be inferred from this data, however, that a correlation existed between these dressings (series 2, 7, 8, 9, 10, 11) and the degree of infestation of the oats in 1937, and that, of the treatments given, calcium cyanamide (series 2) was the most successful in protecting the plants from invasion of their root tissues by the nematode since, in this instance, the cyst population had only doubled. A close analysis of the cyst census for the two seasons (Table I, columns 11 and 12), and consideration of the effect of the treatments on the growth made by the plants in 1937 (p. 863), seemed to provide definite evidence that the variations in the rate of increase in the cyst infestation in 1937 were not bound up with any protection afforded

by the materials added to the soil in 1936 but rather, on the one hand, with the original degree of infection of the soil in the respective series (nos. 2, 7, 8, 9, 10 and 11) as indicated by the cyst counts on the roots in 1936 (Table I, column 11) and, on the other hand, by the amount of growth made by the oat plants of 1937. In all instances the rate of increase in the numbers of cysts in 1937 was inversely proportional to the eelworm infestation of the 1936 oat crop. Thus, of all the treatments, the calcium cyanamide series (no. 2) showed the heaviest root infection in 1936 and the least change in the cyst concentration in 1937. The limitation in the degree of increase in the nematode population imposed in each case was, almost certainly, determined by the extent of the growth made by the plants. That the intensity of eelworm infection may be greatly influenced by the vigour or state of the crop seemed to be well exemplified in the present investigations, both in 1936 (p. 862) and in 1937. In the latter year, in all series (nos, 1, 2, 7, 8, 9, 10, 11) except where oats were grown in soil treated with calcium cyanamide at rates exceeding 20 cwt. per acre (series 3, 4, 5, 6), the growth made by the plants in 1937 was closely similar both in regard to its amount and the symptoms suggesting the presence of "oat sickness" (p. 863). These series also exhibited in the same year remarkable uniformity in the degree of attack by the parasite, the average number of encystic females on the outer roots being in each case approximately 148 per pot (Table I, final column).

6. Summary

An account is given of pot experiments carried out in triplicate in 1936 and 1937 upon the control of the oat sickness associated with the Root eelworm, *H. schachtii*. Calcium cyanamide, sodium nitrate, ferrous sulphate, ferric chloride and two forms of ferric oxide were incorporated intimately with infected soil, and quantitative observations were made on the effects of the treatments upon oats grown in the soil in 1936 and 1937 with regard to (*a*) germination, (*b*) vegetative growth, (*c*) panicle production and yield of grain, and (*d*) degree of infestation of the roots by the nematode. The data obtained show that while all the dressings produced better growth of the plants, at least in the first year after application, calcium cyanamide alone yielded significant results. Further, of the treatments tested, only the use of calcium cyanamide at the rate of as much as 100 cwt. to the acre protected the plants completely from oat sickness in both years and, at the same time, apparently eradicated the parasite from infected soil. Unfortunately, such heavy dressings are not a practicable proposition except, perhaps, under special circumstances.

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EXPLANATION OF PLATE XXXVI

Fig. 1. (*a*) Root system of oat plant from pot receiving calcium cyanamide at the rate of 60 cwt. per acre (series 4, 1936). (*b*) Root system of oat plant from control series, 1936. Note abnormal thickening of roots and excessive development of lateral rootlets.

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a

b

EDWARDS—INVESTIGATIONS UPON THE CONTROL OF OAT SICKNESS BY THE ADDITION OF CERTAIN CHEMICAL SUBSTANCES TO SOIL INFECTED WITH *HETERODERA SCHACHTII* SCHMIDT (pp. 855–866)

NOTE

A NOTE ON CERTAIN VIRUSES OF THE *CUCUMBER VIRUS* 1 TYPE ISOLATED FROM MONOCOTYLEDONOUS PLANTS

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(With Plate XXXVII)

This note records the isolation of viruses of the *cucumber virus* 1 (J. Johnson) type from lily, hyacinth and tulip, and indicates certain differences between the viruses obtained from these monocotyledonous plants and the type virus as determined by the reactions of young tobacco (*Nicotiana tabacum* var. White Burley) and cucumber (*Cucumis sativus* var. Butcher's Disease Resister) plants.

Lily

In April 1937 plants of "*Lilium formosum*" (*L. longiflorum* var. *insulare*) showing typical mosaic mottling and necrotic flecking of the leaves and severe malformation of the flowers were examined and, following the suggestion of Price (1937*a*) that the virus of lily mosaic was related to *cucumber virus* 1, tobacco and cucumber plants were inoculated. A virus of the cucumber mosaic type was detected, and the findings of Price (1937*b*) received general confirmation. Subsequently the same virus was obtained from samples of mosaic diseased "*L. formosum*", from other localities, and "*L. harrisi*" (*L. longiflorum* var. *eximium*).

The lily virus causes circular, pale green or yellowish local lesions (sometimes delimited by fine necrotic lines but never wholly necrotic) on tobacco leaves 4–7 days after inoculation. At first the lesions are often inconspicuous but, as the leaf ages, they become surrounded by dark green bands and more prominent (Pl. XXXVII, fig. 1). Frequently, the virus remains localized in the inoculated leaves, but occasionally systemic infection results, rarely in a first transfer from lily, but more commonly after several transfers through tobacco, when either localized areas of the upper leaves become infected or a more general mild mottle results.

In cucumber faint circular, yellow local lesions, sometimes necrotic on the cotyledons, have been the only symptom, except in one instance when systemic infection occurred. Transfers from systemically infected tobacco gave strains which regularly became systemic in tobacco, but caused a local reaction only on cucumber. The relationship of the lily virus to *cucumber virus* 1 was proved by the double inoculation of *Zinnia elegans* as described by Price.

Hyacinth

A very similar virus to that obtained from lily was isolated from a single hyacinth plant of unknown variety, from Hampshire, which showed a bright yellow streaking of the leaves. The hyacinth virus gives local lesions on tobacco of a similar type to those caused by the lily virus, but rather more severe and usually surrounded by

necrotic bands, 1 mm. or more in width. Systemic infection has occurred on two occasions, as localized infection of upper leaves and once in a more general form. On cucumber, yellow local lesions, but more severe than those caused by the lily virus, have been the only reaction.

Tulip

A third strain of cucumber mosaic virus has been isolated from tulips, variety Zimmerman's Triumph, obtained from two localities in Middlesex by Mr W. Buddin to whom the writer is indebted for the hyacinth plant and other material mentioned in this note). These showed pale yellow or silver-coloured streaks on the leaves (which were malformed), a stunting of the plant and aborted flowers (Pl. XXXVII, fig. 2).

The tulip strain is characterized by the local necrotic reactions of the test plants. In tobacco, 48 hours after inoculation, local lesions appear as pale, green, sunken spots, up to 2 mm. in diameter, which in a few hours turn brown and later become surrounded by a pale green ring (Pl. XXXVII, fig. 3 (*a*)). In cucumber, local lesions are first apparent after 2–3 days as circular dark green spots with a minute necrotic centre but, later, the spots become necrotic and adjacent lesions coalesce (Pl. XXXVII, fig. 4). No systemic infection of any kind has been observed and the virus has, during a period of 3 months, been subjected to ten successive transfers through tobacco without any change in its reaction. The relation of the tulip strain to the type virus has been demonstrated by the suppression of local lesions on tobacco leaves invaded by the latter virus. (In Pl. XXXVII, fig. 3 (*b*), five local lesions caused by the tulip virus are visible in areas not yet invaded by the type virus. In the series from which the photographed leaf was taken 620 lesions developed on the untreated half-leaves, and 40 on the previously inoculated halves.)

Discussion

The three strains of cucumber mosaic virus noted above show a common tendency to remain localized in tobacco and cucumber and, in this respect, differ from the type virus and most of its variants which become systemic in these hosts. The tulip strain, however, shows a similarity to *Strain* 6 described by Price (1934). The intensity of the necrotic action, which appears to be correlated with the tendency to remain localized, is greatest in the tulip and least in the lily virus, and the three strains could legitimately be classified into two groups, the lily and hyacinth strains being more similar to each other than they are to the tulip strain.

Particular interest is attached to the tulip strain on account of McWhorter's (1937) results showing that one of the viruses responsible for tulip break is related to those infecting lilies. Inoculations have been made to tobacco from the flowers, leaves and bulbs of five samples of tulips (Wm. Pitt, Wm. Copland and Clara Butt) showing various degrees of breaking, but no definite evidence has been obtained of the presence of a virus similar to the one detected so readily in Zimmerman's Triumph.

Inoculation experiments are necessary to prove whether the viruses isolated will cause the diseases in the hosts from which they were obtained, and to elucidate their relationships by cross inoculations. It is hoped that such experiments will be carried out when suitable virus free stocks of plants have been assembled.

No evidence has been obtained that viruses of the cucumber mosaic type are implicated in bulbous *Iris* mosaic, *Narcissus* stripe, *Hippeastrum* mosaic and *Gladiolus* mosaic (probably the disease observed by Dosdall (1928)), while the strains of *cu-*

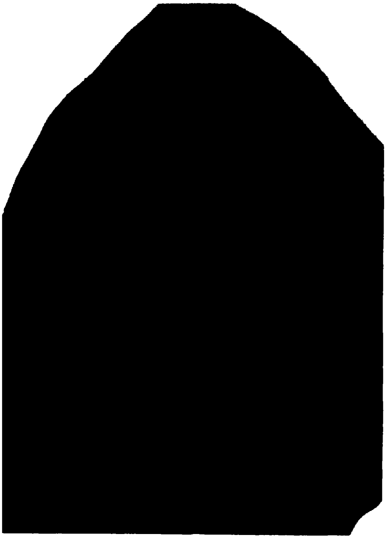

Fig. 1.

Fig. 2

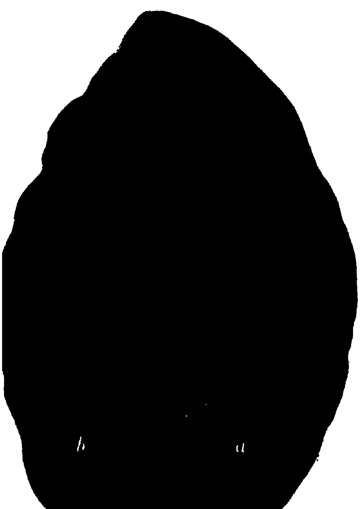

Fig. 3.

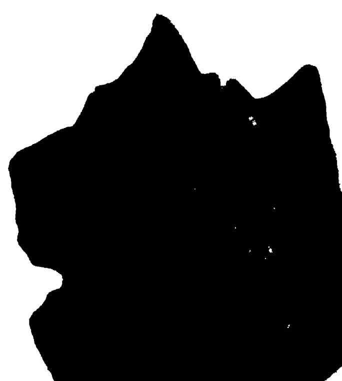

Fig. 4.

AINSWORTH.—A Note on Certain Viruses of the *Cucumber virus* 1 Type Isolated from Monocotyledonous Plants (pp. 867–869)

cumber virus 1 isolated by the writer during the last few years from dicotyledonous plants belonging to several different families have all approximated to the type virus.

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EXPLANATION OF PLATE XXXVII

Fig. 1. Local lesions on tobacco caused by Lily strain (25 days).

Fig. 2. Diseased tulip, variety Zimmerman's Triumph.

Fig. 3. Tobacco leaf 7 days after inoculation with the tulip strain, 11 days after left-hand half of leaf (*b*) inoculated with type *cucumber virus* 1.

Fi . 4. Local lesions on cucumber caused by tulip strain (19 days).

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REVIEWS

Forest Pathology. By J. S. Boyce. Pp. x + 600. London: McGraw-Hill Publishing Co., Ltd. 1938. 30*s.* 0*d.*

Following a brief introductory chapter, Ch. II contains a short discussion of the nature, symptoms and terminology of plant disease, and Ch. III a brief outline of the fungi based on Heald's presentation but emphasizing the Basidiomycetes. Ch. IV deals shortly with the non-infectious diseases of trees, the author remarking that "few of them have been carefully studied" and "the aggregate damage they cause to the forests of this country is not great". Ch. V is devoted to seedling diseases and Ch. VI to root diseases—including mycorrhiza and root nodules. Chs. VII and VIII deal, respectively, with foliage diseases of hardwoods and of conifers. Chs. IX–XVII are devoted to stem diseases as follows: Rusts of conifers; White pine blister rust; Cankers of conifers; Cankers of hardwoods; Galls and witches' brooms; Diebacks and wilts; Mistletoes, epiphytes and climbers; Decay; Rots. Chs. XVIII–XX are concerned with deterioration of forest products: decay in timber; decay in wood; sap stains. Ch. XXI outlines the general principles of forest-disease control. Appendix A contains brief directions for making fungicides and Appendix B a list of common and scientific names of trees. Terminating the chapters are useful bibliographies, and the book concludes with a good index.

The only comparable recent work in English is Hubert's *Outline of Forest Pathology*, 1931, which deals with the same range of material, and is of approximately equal size and calibre. The two volumes resemble each other in their general treatment of introductory matter and of timber and wood decay, but differ in the arrangement of the parasitic diseases of trees. Prof. Boyce arranges his material on the basis of the parts of the tree affected and the tree species, whilst Prof. Hubert bases his arrangement on the taxonomic sequence of the causal organisms, prefacing his consideration by detailed keys of symptoms. Prof. Boyce states that his experience has convinced him that his method is the better, and gives his reasons, whilst Prof. Hubert would, undoubtedly make an equally good case.

A similar problem faces anyone who attempts to teach plant pathology. My own experience has been with students of agriculture and horticulture, and I have tried out the two systems alternately over a number of years. I have come definitely to the conclusion that the arrangement of material by taxonomic sequence of parasites, combined with summarizing reviews of diseases arranged on the plant organ and host basis, is the better. The ordering of material in this way affords not only a more logical presentation of the subject but appears to be much more convincing to students, even those of essentially practical type. The danger is that such courses may become more mycological than pathological in outlook. The organ and host arrangement, in my experience, tends to lead to an empirical and rule of thumb attitude on the part of students, whereas the taxonomic arrangement is equally successful as a method of presenting data and yet emphasizes principles. In the study of plant disease, the essential thing is not a knowledge of a great number of empirical data, but a knowledge of pertinent data, combined with an understanding of principles and a capacity to adapt these to particular cases.

Dr Boyce has compiled and selected his material very conscientiously and, within the general plan, the detailed arrangement is excellent. The text is clear and simple as befits a text-book, but, although it is difficult to say just why, the author's style of writing is curiously dull and uninspired. There are quantities of sentences such as "Fall sowing in northern nurseries where the soil remains practically continuously frozen is less liable to result in loss than spring sowing, although weather is so variable as to time that this method of control is somewhat uncertain" or "Probably soil

character has been affected by the elimination of chestnut, but whether favourably or unfavourably it is difficult to surmise". Having read pages of this sort of thing one feels quite cheered by coming across so charming a name as "the weeping conk", such a sequence as "paintbrush, lousewort, owl's-clover, and bird's beak", or such a delicious sentence as "Individual witches' brooms also die frequently".

The book is illustrated by 216 text-figures, many of which are original, and few of which state the magnification. The only misprint noted is in the legend to Text-fig. 202.

WILLIAM B. BRIERLEY.

Yearbook of Agriculture, 1937. Pp. vi + 1497. United States Department of Agriculture, Washington, D.C. $2·00.

This volume concludes the survey of animal and plant improvement begun in the Yearbook, 1936 (see *Ann. appl. Biol.* 1937, **24**, 679), which dealt with the genetics and breeding of cereals, sugar plants, fibre plants, tobacco, cattle, swine, sheep, horses and mules, and poultry.

The *Yearbook*, 1937, covers an enormous and varied field dealing with: Vegetables—general (5 pp., Boswell); Tomato, Pepper, and Eggplant (31 pp., Boswell); Onions (26 pp., H. A. Jones); Peas and Beans (32 pp., Wade); Leafy cruciferous vegetables (17 pp., Magruder); Root vegetables (26 pp., Poole); Salad crops (14 pp., Thompson); Vegetable crops—appendix (39 pp., Boswell); Sweet corn (16 pp., Poole); Popcorn (10 pp., Brunson); Potatoes (40 pp., Stevenson & Clark); Strawberry (51 pp., Darrow); Blackberry and Raspberry (38 pp., Darrow); Currants and Gooseberries (11 pp., Darrow); *Actinidia* spp., *Viburnum* spp., *Elaeagnus* spp., Quince, *Prunus* spp. (14 pp., Darrow & Yerkes); Blueberry (16 pp., Coville); Apple (40 pp., Magness); Pear (16 pp., Magness); Grape (34 pp., Snyder); Stone fruits (84 pp., Cullinan); Subtropical fruits, *Citrus* (78 pp., Traub & Robinson); Nuts (63 pp., Crane, Reed & Wood); Flowers (109 pp., Emsweller, P. Brierley, Lumsden & Mulford); Miscellaneous forage and cover crop legumes (33 pp., McKee & Pieters); Miscellaneous grasses (71 pp., Vinall & Hein); Timothy (18 pp., Evans); Alfalfa (32 pp., Tysdal & Westover); Soybean (36 pp., Morse & Cartter); Clover (25 pp., Pieters & Hollowell); Hop (27 pp., D. C. Smith); Forest trees (38 pp., Schreiner); Angora goats (14 pp., Lambert); Milk goats (21 pp., Simmons & Lambert); Dogs (35 pp., Dawson); Turkeys (17 pp., Marsden & Knox); Ducks (12 pp., Lee); Fur animals (17 pp., Ashbrook); Bees (23 pp., Nolan). There are also the following more general articles: Fundamentals of heredity for breeders (31 pp., Bressman & Hambidge); Vegetative reproduction (7 pp., Magness); Chronology of genetics (22 pp., Cook). The volume opens with a useful summary of the articles, "What the book is about" (52 pp., Hambidge) and concludes with a good index. Certain other papers and extensive bibliographies, which complete and round off the survey, have been excluded by lack of space, but are stated to be available as *Yearbook* separates.

Together, the genetical portions of the two *Yearbooks* total 2450 well-illustrated pages, containing 69 articles, and they form by far the most comprehensive treatise on animal and plant breeding yet published in the English language. Nothing is of more vital concern to farmers and growers than the improvement of plants and animals, or more valuable to students than an authoritative source book of viewpoints and data. To write for both practical farmers and students is an exceedingly difficult task, but most of the authors have succeeded in presenting their material in reasonably non-technical language which is yet accurate in form and substance.

The articles themselves vary considerably in quality but, on the whole, they are complete and up-to-date. They are written by Americans for Americans and, thus, deal primarily with animals and plants important in American farming, but the United States comprises so many and varied conditions that the volumes are international in content and outlook. Bibliographies citing work referred to in the text are usually a fair guide to the writers' width of view. The bibliography to, for example, Stevenson & Clark's paper on potato breeding, cites 26 American works, 16 British, 13 Russian, 13 German, 2 Scandinavian, 2 Japanese, and 2 French; whilst that to

e.g. Dawson's paper on heredity in the dog, cites 22 American works, 18 British, 9 German, 5 Scandinavian, 4 Russian, 3 French, 2 Swiss, and 1 each Dutch, Polish, Italian, and Japanese; and these bibliographies are typical.

The volumes contain not only an account of what has been done and is being done to improve plants and animals in the U.S.A. They are, also, an attempt to make a frank appraisal of the present situation—not only to sum up achievements, but to expose weaknesses and shortcomings. The achievements of American breeders are magnificent and, because of them, entire fields of American agriculture have been remodelled. There are, however, regions of agricultural practice where modern genetical knowledge and technique are still largely unapplied or where breeding is, at best, largely empirical. In writing of such problems the authors consider the possibilities and have tried to foresee fruitful lines of enquiry.

The survey was organized by a Committee on Genetics appointed in 1933 by the U.S. Secretary of Agriculture, Mr Henry A. Wallace, himself not only a distinguished public servant but a plant breeder for some 30 years. To each volume he has contributed a foreword and extracts from these may fittingly close this notice. "I trust that the day will come when humanity will take as great an interest in the creation of superior forms of life as it has taken in past years in the perfection of superior forms of machinery. In the long run superior life forms may prove to have a greater profit for mankind than machinery". "Man's control of his future may depend in the long run on whether his biological knowledge, which is constructive, can catch up with his knowledge of the physical sciences, which has taught him so much about how to destroy".

WILLIAM B. BRIERLEY.

Physiological Genetics. By R. GOLDSCHMIDT. Pp. ix + 375. London: McGraw-Hill Publishing Co., Ltd. 1938. 24*s*. 0*d*.

The author has endeavoured "to present the entire material available", and "to organize it into the skeleton of a future science of physiological genetics". The book is written in a technical and somewhat involved style, and knowledge of genetics and embryology is assumed on the part of the reader.

The work is arranged in four sections, and terminates with a useful bibliography of 45 pages, and author and subject indexes. Section I is an introductory note contrasting static genetics with dynamic or physiological genetics.

Section II (260 pages) contains a detailed consideration of the mutated gene and the potentialities of development. The general thesis underlying the author's treatment is that an understanding of the action of the gene can only be obtained by comparing the effects of the mutated gene upon development with effects produced by external agencies upon the development of the Wild type. Incorporating a wealth of data from plant and animal studies, the author builds up a coherent and logical scheme of the genetics of development processes based on the concept of genes as affecting rates. An interesting omission is any reference to Garrod's work.

Section III (20 pages) deals with the role of the cytoplasm in heredity. The author concludes that whilst the cytoplasm is the substratum for genic action, and its specificity one of the prerequisites of orderly development, no specific hereditary traits exist that are transmitted through the cytoplasm and are individually caused by a genetic property of the cytoplasm. Plant plastids are regarded as a third independent constituent of the cell in regard to heredity.

In Section II the existence of the gene is taken for granted, since the data can be considered independently of conceptions regarding its nature. In Section IV (35 pages), the nature of the gene is discussed and, to readers who do not specialize in genetics, these pages will be as interesting as they are controversial. In Prof. Goldschmidt's view "The developments in the last years of genetical research have now led to a point where it has become necessary to ask the radical question: Is not the whole conception of the gene as a hereditary unit obsolete?" He answers this question in the affirmative, considering that the evidence strongly suggests "That the chromosome is the actual hereditary unit controlling the development of the Wild type, that

purely steric changes at the individual points of its length produce deviations from the Wild type which may be described as mutations, even as point mutations, though no actual Wild type allelomorph and therefore no gene exists." This means, more simply, that chromosomes are physiological wholes, and that genes are spatial internal rearrangements of parts of them, which alter their functions. To replace the gene theory the author postulates a chemical theory of heredity, supporting this by the work of Bergmann, Koltzoff, Wrinch, etc., and remarks "It remains for the physico-chemist to decide whether or not the new model could also take care of the independent catalytic actions of what was considered to be a gene."

WILLIAM B. BRIERLEY.

The Study of Heredity. By E. B. FORD. Pp. 256. London: Thornton Butterworth. (Home University Library, Vol. 187.) 1938. 2*s.* 6*d.*

This book is a first-class introduction to the subject for any intelligent person who will apply himself seriously to its study. The chapters run: The physical basis of heredity; The laws of inheritance; The inheritance of sex; Mutation; The nature of heredity; Variation; The action of genetic factors; Selection; Practical applications, and human heredity; Evolution. The book closes with a glossary, a bibliography of sixteen annotated references, and an index.

The author writes in a clear but condensed style, examples are drawn primarily from the animal kingdom, and his treatment of the subject has been influenced largely by the work and views of Darlington, Fisher, Haldane, and Huxley. In discussing human heredity the author makes statements which might not be accepted by all workers. The book is so up-to-date that, even four months after its publication, two of the volumes cited in the bibliography have not yet appeared. Among the other books suggested for further reading are one or two which are perhaps rather out-of-date, one which is very stiff going for first-class Honours students, and one which contains whole paragraphs admitted by professors of the subject to be unintelligible.

WILLIAM B. BRIERLEY.

Tropical Fruits and Vegetables: an account of their Storage and Transport. By C. W. WARDLAW. Pp. xii+224. Imperial College of Tropical Agriculture, Trinidad, B.W.I.; Low Temperature Research Station, Mem. No. VII. 1937. 4*s.* 0*d.*

This bulletin comprises articles, originally published in *Tropical Agriculture*, 1937, **14**, nos. 3–12, dealing with the storage and transport of some seventy-four fruits and vegetables indigenous to, or capable of being grown in, the tropics. Attention is chiefly restricted to the major practical issues. The arrangement is alphabetical under the common names of the plants, and to each is appended a good bibliography. A rather unexpected thing is the number of plants included which are usually regarded as being crops of temperate or warm temperate lands: beans and peas, beetroot, various common Brassicas, carrot, celery, filbert, lettuce, radish, spinach, water-cress. Tropical crops, naturally, receive the greatest consideration, special attention being given to avocado, banana, *Citrus*, mango, onion, papaw, persimmon, pineapple, potato, sweet potato, tomato.

The tomato may be taken as an example of the author's treatment: the fruit is discussed in 26 pages under the following heads: Introductory; General considerations; Chemical changes during development and ripening; Pre-storage factors and quality (colour development, effects of fertilizers and soil moisture, temperature of growth, late harvesting, disease incidence); Harvesting maturity; Debuttoning; Packing-shed treatments (disinfectant treatment, standardization of packing maturity, culling and grading for size and colour, wrapping and packing); Transport by rail; Rapid cooling;

Interruption of cold storage; Storage and ripening temperatures (storage at low and higher temperatures, experience in various countries, storage of tropically grown fruit); Low temperature injury (symptoms of chilling); Loss in weight; Effect of light on ripening; Sulphur dioxide injury; Ethylene ripening; Gas storage; 64 references.

Much of the literature on this subject is scattered in journals which, often, are not easily available and this bulletin, which seems to have been compiled carefully, is a very useful publication.

WILLIAM B. BRIERLEY.

Erosion and Soil Conservation. By G. V. JACKS and R. O. WHYTE. *Herbage Publication Series, Bull.* No. 25. Pp. 206. Aberystwyth: Imperial Bureau of Pastures and Forage Crops. (Also published as *Technical Communication* No. 36 from the Imperial Bureau of Soil Science, Harpenden.) 1938. 5*s*.

Soil erosion is one of the most disquieting features in world agriculture today and, in many countries, the capital loss is taking place with a rapidity and on a scale which, in any other basic industry, would lead to immediate national action. Although it has reached almost catastrophic proportions soil erosion is not, like an earthquake, an "act of God": it is an entirely preventible process, the principles of soil conservation are fairly well established and it is imperative that they become widely known and applied before the destruction becomes even more calamitous. A first essential is an accurate survey of the present position, so that conditions and trends and methods of control and regeneration in various lands may be compared. In compiling this account of contemporary soil erosion, the authors have not only consulted widely in the literature of the subject but have had the active cooperation of correspondents and workers throughout the world, and this has enabled them to present a brief but authoritative picture of conditions in the Mediterranean region, U.S.S.R., India, Ceylon, East Indies, China, Japan, French overseas possessions, South Africa, Rhodesia, East Africa, West Africa, U.S.A., Canada, West Indies, Australia, and Fiji. The bulletin contains a mass of information and is well put together but a useful addition would have been a concluding section summarizing and comparing conditions and developments in the various countries. This bulletin should receive the widest circulation possible among not only agricultural scientists but also sociologists and administrators.

WILLIAM B. BRIERLEY.

Sixty Years of Botany in Britain (1875–1935). *Impressions of an Eye-witness.* By F. O. BOWER. Pp. vii + 112. With frontispiece and 13 other illustrations. London: Macmillan and Co., Ltd. 1938. 10*s*. 6*d*.

Prof. F. O. Bower was born four years before the publication of the *Origin of Species* and, during 60 years bridging the nineteenth and twentieth centuries, he was one of the international figures in botanical science. In that period he came to know intimately most of the men who, with himself, helped to make botanical history.

The autobiographical chapters opening the book give a vivid impression of the conditions of botanical study in the 1870's and 1880's, conditions which would seem hardly credible to modern students who take fully equipped laboratories for granted. In a series of admirable character sketches accompanied by excellent portraits, the author introduces the men who brought about these changes, names familiar to every student of botany. A short and rather more technical chapter gives a comprehensive survey of the changes of outlook on the vegetation of the land during the period since 1875 and these pages, which are really an epitome of Prof. Bower's

life-work, are a masterly piece of writing. In a final chapter the author attempts to relate the botanical events of 60 years to the broad stream of scientific progress.

The illustrations form a magnificent gallery of portraits and one's only regret is that Prof. Bower's modesty has excluded his own.

WILLIAM B. BRIERLEY.

Herbals: Their Origin and Evolution. A Chapter in the History of Botany, 1470–1670. By AGNES ARBER. 2nd edition. Pp. xxiv + 326. Cambridge: University Press. 1938. 21*s*. 0*d*.

Mrs Arber has rewritten and enlarged her volume on *Herbals*, of which the first edition (1912) has long been out of print. The general plan of the book remains unaltered, but newer knowledge has enabled her to fill in many interesting details and to add sections dealing with botany in Spain and Portugal, and with the origin of herbaria. The illustrations have been increased by 5 plates and 18 text-figures. Appendix I has been enlarged, Appendix II trebled, and a third appendix, a subject-index to Appendix II, has been added.

In 1934 Mrs Arber published her magistral work on *The Gramineae*, and in reviewing this (*Ann. appl. Biol.* 1935, **22**, 433), I said: "Permeating the whole book is a certain fragrance, a controlled discursiveness and wide-ranging quality that one always associates with the great herbalists of a bygone age. It is a style of writing almost unique in the professionalized botany of to-day and it is a delight to read." Mrs Arber has now written of the great herbalists themselves. It is a subject she has made peculiarly her own, and in which her sympathetic vision, and her charming and scholarly style find natural expression and full scope. Her book is pure gold. Those who treasured the old edition will find increased delight in the new, although they may regret the passing of the familiar covers, whilst those who now meet the book for the first time have an enviable joy in store.

WILLIAM B. BRIERLEY.

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1930 Dawson, R. B., M.Sc., F.L.S., Director, St Ives Research Station, Bingley, Yorkshire.

1936 Dennis, R. W. G., B.Sc., Ph.D., Seed Testing and Plant Registration Station, East Craigs, Corstorphine, Edinburgh, 12.

1932 Dinkley, Mrs D., M.Sc., The Priory, Berwick St John, Shaftesbury, Dorset.

1923 Dixon*, Miss A., M.Sc., F.R.M.S., Kauguri, Batchwood Drive, St Albans, Herts.

1920 Dowson, W. J., M.A., D.Sc., Botany School, Cambridge. (Council 1937– .)

1937 Dowson, Mrs M. J. 26 Barrow Road, Cambridge.

1920 Drummond, Prof. J. M., M.A., F.L.S., Department of Botany, The University, Manchester.

1935 Duffield, C. A. W., F.R.E.S., The Cooper Technical Bureau, 47, Russell Square, London, W.C. 1.

1923 Du Porte, Ernest Melville, M.Sc., Ph.D., F.R.E.S., F.R.M.S., Macdonald College, Montreal, Canada.

1928 Eastham, Prof. L. E. S., M.A., M.Sc., Department of Zoology, The University, Sheffield.

1927 Edwards, E. E., M.Sc., Department of Agricultural Zoology, University College, Cardiff.

1935 Ekins, Miss E. H., B.Sc., Principal, The College, Studley, Warwickshire.

1934 Engledow, Prof. F. L., M.A., C.M.G., School of Agriculture, Cambridge.

1922 Esdaile, Miss P. C., D.Sc., F.Z.S., King's College of Household and Social Science, Campden Hill Road, London, W. 8.

1935 Evans, A. C., B.Sc., D.I.C., Rothamsted Experimental Station, Harpenden, Herts.

1927 Everett, J., B.A., Canterton Cottage, Lyndhurst, Hants.

1920 Fahmy, T., D.Sc., Mycological Division, Plant Protection Section, Ministry of Agriculture, Giza, Cairo, Egypt.

1920 Fenton, E. Wyllie, M.A., B.Sc., F.R.E.S., Biological Department, Edinburgh, and East of Scotland College of Agriculture, 13, George Square, Edinburgh.

1937 Ferguson, R. G., M.Agr.Sc., Herts. Institute of Agriculture, Oaklands, St Albans, Herts.

1937 Fidler, J. H., B.A., Ph.D., Dept. of Agricultural Entomology, Victoria University, Manchester, 13.

1929 Findlay, W. P. K., M.Sc., A.R.C.S., D.I.C., Forest Products Research Laboratory, Princes Risborough, Bucks. (Botanical Secretary, 1936, General and Botanical Secretary, 1937– .)

1935 Fish, S., Department of Agriculture for Victoria, Public Offices, Melbourne, Victoria, Australia.

1919 Fisher, K., The School, Oundle, Northampton.

1923 FISHER, R. C., B.Sc., Ph.D., Forest Products Research Laboratory, Princes Risborough, Bucks. (Vice-President, 1936; Council, 1934–1936.)

1931 FOISTER, C. E., B.A., Ph.D., Royal Botanic Garden, Edinburgh.

1920 FOX-WILSON, G., N.D.Hort., F.L.S., F.R.E.S., R.H.S. Laboratories, Wisley, Ripley, Surrey. (General and Zoological Secretary, 1935–36; Zoological Secretary, 1933–1934; 1937- ; Council, 1927–1929.)

1936 FRANKLIN, Miss M. T., B.Sc., Institute of Agricultural Parasitology, Winches Farm, St Albans, Herts.

1913 FRYER, J. C. F., O.B.E., M.A., F.R.E.S., Pathological Laboratory, Milton Road, Harpenden, Herts. (President, 1926–1927; Treasurer, 1914–1919; Council, 1921, 1924–1925.)

1933 GADD, C. H., D.Sc., Tea Research Institute, St Coombs, Talawakelle, Ceylon.

1930 GALLOWAY, L. D., M.A., 31 Lake Close, Wimbledon, S.W. 19.

1935 GARRETT, S. D., M.A., Rothamsted Experimental Station, Harpenden, Herts.

1936 GIBBONS, J., M.Sc., Avoncroft, Stoke Prior, Bromsgrove, Worcs.

1927 GIBSON, W. H., O.B.E., D.Sc., F.I.C., F.Inst.P., Director, The Linen Industry Research Association, The Research Institute, Lambeg, Co. Antrim, Northern Ireland.

1938 GILL, N. T., B.Sc., Ph.D., Midland Agricultural College, Sutton Bonington, Loughborough.

1937 GILLESPIE, J., B.Sc., Botany Dept., The University, Reading.

1937 GILLESPY, T. G., B.Sc., A.R.C.S., University of Bristol Fruit and Vegetable Preservation Research Station, Campden, Glos.

1920 GIMINGHAM, C. T., O.B.E., F.I.C., F.R.E.S., Pathological Laboratory, Milton Road, Harpenden, Herts. (Zoological Editor, 1934– ; Council, 1929–1932; Vice-President, 1937; President, 1938- .)

1920 GLYNNE, Miss M. D., M.Sc., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.

1920 GOODEY, T., D.Sc., Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St Albans. (President, 1934–1935; Council, 1923–1929; 1936–1938.)

1929 GRAINGER, J., Ph.D., Tolson Memorial Museum, Ravensknowle, Huddersfield.

1921 GRAY, Prof. P. H. H., M.A., Macdonald College, Quebec, Canada.

1929 GREEN, D. E., M.Sc., R.H.S. Laboratories, Wisley, Ripley, Surrey.

1935 GREEN, T. L., Dept. of Zoology, The University, Bristol.

1933 GREGOR, J. W., Ph.D., Scottish Plant Breeding Station, Craigs House, Corstorphine, Edinburgh.

1934 GRIEVE, B. J., M.Sc., Ph.D., D.I.C., Botany School, University of Melbourne, Victoria, Australia.

1921 GRUBB, N. H., M.Sc., East Malling Research Station, East Malling, Kent.

1934 GÜSSOW, H. T., F.L.S., F.R.M.S., Dominion Botanist, Central Experimental Farm, Ottawa, Canada.

1920 GWYNNE-VAUGHAN, Prof. Dame HELEN, D.B.E., D.Sc., LL.D., F.L.S., Birkbeck College, Chancery Lane, London, E.C. 4.

1920 HALKET, Miss A. C., B.Sc., Bedford College, Regent's Park, London, N.W.

1930 Hall, W. J., D.Sc., A.R.C.S., F.R.E.S., Director, Citrus Experimental Station, P.O. Mazoe, S. Rhodesia, Africa.

1924 Harris, R. V., A.R.C.S., B.Sc., East Malling Research Station, East Malling, Kent.

1936 Hatt, W. E., Garden Fertilisers Ltd., Nelson Road, Wanstead, London, E. 11.

1938 Harvey, G. L., Asiatic Petroleum Co. Ltd., Great St Helens, London, E.C. 3.

1927 Hatton, R. G., C.B.E., M.A., D.Sc., Director, East Malling Research Station, East Malling, Kent. (Council, 1928.)

1938 Henderson, F. Y., D.Sc., Imperial College of Science, London, S.W. 7.

1935 Herford, Miss G. M., B.Sc., 1, Frognal Gardens, Hampstead, N.W. 3.

1933 Hey, G. L., M.A., c/o The Murphy Chemical Co., Wheathampstead, Herts.

1920 Hill, Sir A. W., K.C.M.G., M.A., Sc.D., F.R.S., F.L.S., Director, Royal Botanic Gardens, Kew, Surrey.

1920 Hiscox, Miss E. R., B.Sc., National Institute for Research in Dairying, Shinfield, Nr. Reading.

1936 Hobson, R. P., B.Sc., Ph.D., School of Agriculture, University College of North Wales, Bangor.

1924 Hockey, J. F., B.S.A., Pathologist in Charge, Plant Pathology Laboratory, Kentville, Nova Scotia, Canada.

1935 Hodson, W. E. H., A.R.C.S., D.I.C., The University, Reading.

1937 Holborn, G. M., B.Sc., A.R.C.S., Jeyes Sanitary Compounds, Richmond Street, Plaistow, London, E. 13.

1938 Holman, H. J., B.Sc., A.R.C.S., A.I.C., Imperial Institute, South Kensington, London, S.W. 7.

1919 Horne, A. S., D.Sc., F.L.S., F.G.S., Botany School, Imperial College of Science, London, S.W. 7.

1920 Horton, E., B.Sc., F.I.C., 10, Crieff Road, Wandsworth Common, London, S.W. 18.

1927 Howes, F. N., D.Sc., Royal Botanic Gardens, Kew, Surrey. (Council, 1930–1931.)

1928 Hughes, A. W. McKenny, D.I.C., Natural History Museum, S. Kensington, London, S.W. 7.

1936 Hull, R., B.Sc., A.R.C.S., Midland Agricultural College, Sutton Bonington, Loughborough.

1938 Hunter-Smith, M. B. E., B.Sc., N.D.A., Herts Institute of Agriculture, Oaklands, St Albans.

Orig. Imms, A. D., M.A., D.Sc., F.R.S., F.L.S., F.R.E.S., Zoological Laboratory, The Museums, Cambridge. (President, 1930–1931; Vice-President, 1924–1927; Treasurer, 1920–1930; Council, 1914–1920; 1933–1935.)

1918 Jackson, Miss D. J., F.L.S., F.R.E.S., North Cliff, St Andrews, Fife, Scotland.

1927 Jacobs, S. E., Ph.D., Bacteriology Department, Imperial College of Science, London, S.W. 7.

1927 James, H. C., D.Sc., Zoological Laboratory, The Museums, Cambridge.

1927 Jary, S. G., B.A., S.E. Agricultural College, Wye, Kent. (Council, 1934-1936.)

1934 Johnson, C. G., B.Sc., London School of Tropical Medicine and Hygiene, Gower Street, London.

1938 Johnson, L. R., M.Sc., Department of Agriculture, The University, Leeds 2.

1931 Jones, A. P., M.Sc., Department of Agriculture, The University, Leeds 2.

1934 Jones, E. Parry, B.Sc., Ph.D., A.I.C.T.A., F.R.E.S., Citrus Experimental Station, P.O. Mazoe, S. Rhodesia, Africa.

1927 Jones, G. H., M.A., Plant Protection Section, Ministry of Agriculture, Cairo, Egypt.

1927 Joseph, E. G., B.Sc., Redoot, Swanley, Kent.

1935 Kearns, H. G. H., B.Sc., Ph.D., The Research Station, Long Ashton, Bristol.

1932 Kerr, H. W., Ph.D., M.Sc., Director, Bureau of Sugar Experiment Station, Brisbane, Australia.

1920 Kidd, F., M.A., D.Sc., Director Low Temperature Research Station, Downing Street, Cambridge.

1907 King, H. H., F.L.S., F.R.E.S., Government Entomologist, Wellcome Tropical Research Laboratories, Khartoum, Sudan.

1907 King, Prof. L. A. L., M.A., West of Scotland Agricultural College, 6, Blythewood Square, Glasgow.

1926 Kingston, H. T., Athlone, High Road, Broxbourne, Herts.

1933 Kramer, L. M. J., M.A., Ph.D., City of London School, Victoria Embankment, London, E.C. 4.

1921 Lacey, Miss M. S., M.Sc., Botanical Department, Imperial College of Science, London, S.W. 7.

1935 Ladell, W. R. S., F.I.C., Amity Hall, Alley, P.O. Jamaica, B.W.I.

1921 Laurie, Prof. R. D., M.A., F.Z.S., Department of Zoology, University College of North Wales, Aberystwyth.

1933 Leach, R., B.A., School of Agriculture, Cambridge.

1934 Lean, O. B., B.Sc., D.I.C., Imperial Chemical Industries, Jealott's Hill, Bracknell, Berks.

1926 Le Pelley, R. H., Ph.D., Department of Agriculture, Nairobi, Kenya, Africa.

1920 Lloyd, Llewellyn, D.Sc., Zoological Department, The University, Leeds.

1937 Lovibond, Miss C. B. J., B.Sc., Rothamsted Experimental Station, Harpenden, Herts.

1931 McClean, A. P. D., D.Sc., Natal Herbarium, Berea, Durban, Natal, Union of S. Africa.

1914 McClellan, F. C., C.B.E., M.R.A.C., F.L.S., Director of Agriculture, Zanzibar.

1936 Macdonald, J. A., B.Sc., Ph.D., The University, St Andrews.

Orig. Macdougall*, Prof. R. S., M.A., D.Sc., F.R.S.E., F.R.E.S., Ivy Lodge, Gullane, E. Lothian, Scotland. (Vice-President, 1914–1919; Council, 1908–1913.)

1929 MacGill, Miss E. I., D.Sc., Linkfield, Hawthorn Road, Denton, Manchester.

1927 McLennan, Miss Ethel, D.Sc., Botanical Department, The University, Melbourne, Australia.

1925 Macleod, D. J., M.A., 20 Highworth Avenue, Cambridge.

1938 McLeod, J., D.Sc., Cooper Field Research Station, Little Gaddesden, Herts.

1909 Mangan, Prof. J., M.A., 3, Ceiriog Close, Penarth, Glamorgan, S. Wales.

1920 Mangham, Prof. S., M.A., University College, Southampton.

1917 Mann, H. H., D.Sc., F.L.S., Woburn Experimental Station, Aspley Guise, Bedfordshire.

1938 Marsh, R. W., M.A., Research Station, Long Ashton, Bristol.

1934 Marshall, J., B.Sc., A.R.C.S., A.I.C.T.A., c/o Empire Cotton Growing Corporation, King's Buildings, Dean Stanley Street, London, S.W. 1.

1930 Martin, H., D.Sc., A.R.C.S., Research Station, Long Ashton, Bristol. (Council, 1936–1938; Vice-President, 1938.)

1922 Mason, E. W., M.A., M.Sc., Imperial Mycological Institute, Kew, Surrey.

1920 Mason, F.A., F.R.M.S., 29, Frankland Terrace, Leopold Street, Leeds.

1933 Mason, Miss M., B.Sc., (Hort.), Ministry of Agriculture and Fisheries, 10 Whitehall Place, London, S.W. 1.

1927 Massee, A. M., D.Sc., F.R.E.S., East Malling Research Station, East Malling, Kent. (Council, 1932–1934.)

1920 Mattick, A. T. R., B.Sc., Ph.D., National Institute for Research in Dairying, Shinfield, Nr. Reading.

1938 Meggitt, F. J., M.Sc., Ph.D., University College, Rangoon.

1933 Meiklejohn, Miss J., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.

1932 Metcalfe, Miss M. E., B.Sc., Ph.D., 20, Llantwit Road, Treforest, Glamorganshire.

1923 Miles, H. W., D.Sc., Ph.D., Department of Agricultural Entomology, Victoria University, Manchester. (Council 1937– .)

1921 Millard, W. A., D.Sc., Department of Agriculture, The University, Leeds 2.

1932 Misra, A. B., D.Sc., Ph.D., F.Z.A., F.R.E.S., Department of Zoology, Benares Hindu University, India.

1938 Moore, E. J., M.Sc., Central Technical College, Suffolk Street, Birmingham, 1.

1932 Moore, Miss E. S., B.Sc., Ph.D., Mycologist, Tobacco Research Laboratory, Balfour, C.P. South Africa.

1928 Moore, W. C., M.A., Plant Pathological Laboratory, Milton Road, Harpenden, Herts. (Council 1938– .)

1922 Morland, D. M. T., M.A., Rothamsted Experimental Station, Harpenden, Herts.

1920 Morris, H. M., M.Sc., Agricultural Department, Nicosia, Cyprus.

1920 Mosley, F. O., F.L.S., Pathology Laboratory, Messrs Lowe and Shawyer, Uxbridge, Middlesex.

1914 Munro, Prof. J. W., M.A., D.Sc., Zoology Department, Imperial College of Science, London, S.W. 7. (Vice-President, 1933; General and Zoological Secretary, 1928–1932; Zoological Editor, 1933; Council, 1922–1924, 1927.)

1919 Murphy, A. J., Technical Chemist, The Murphy Chemical Co., Wheathampstead, Herts.

1920 Murphy, Prof. P. A., Sc.D., B.A., Albert Agricultural College, Glasnevin, Dublin, Irish Free State.

1938 Musgrave, A. J., B.Sc., D.I.C., Imperial College of Science and Technology, London, S.W. 7.

1925 Muskett, A. E., B.Sc., A.R.C.S., D.Sc., The Queen's University, Belfast, N. Ireland.

1937 Narasimhan, M. J., B.A., Mycologist, Dept. Agriculture, Bangalore Mysore State, India.

1937 Nattrass, R. M., Ph.D., Dept. of Agriculture, Nairobi, East Africa.
Newport, Salop. (Council 1937– .)

1928 Nel, R. I., B.Sc., M.Sc., Naga Hoeta Estate, Pematang Siantar, Sumatra, (East Coast).

1928 NEWTON, H. C. F., Ph.D., A.R.C.S., D.I.C., Harper Adams Agricultural College, Newport, Salop. (Council, 1937-)

1930 NORMAN, A. C., D.Sc., F.I.C., Sails Dept., Iowa State College, Ames, Iowa, U.S.A.

1921 NOWELL, Wm, D.I.C., F.L.S., Director, East African Agricultural Research Institute, Amani, Tanga, Tanganyika Territory, E. Africa.

1928 NUTMAN, F. J., B.Sc., A.R.C.S., East African Agricultural Research Station, Amani, Tanga, Tanganyika Territory, E. Africa.

1923 OGILVIE, L., M.A., M.Sc., Research Station, Long Ashton, Bristol.

1925 OLDHAM, J. N., B.Sc., Ph.D., "Heriots", 336 Hatfield Road, St Albans, Herts.

1937 OYLER, Miss ENID, B.Sc., Experimental and Research Station, Cheshunt, Herts.

1928 PARKIN, E. A., B.Sc., Ph.D., Forest Products Research Laboratory, Princes Risborough, Bucks.

1935 PEARL, R. T., B.Sc., A.R.C.S., South Eastern Agricultural College, Wye, Kent.

1914 PETHERBRIDGE, F. R., M.A., School of Agriculture, Cambridge.

Orig. PETHYBRIDGE, G. H., M.A., M.R.I.A., Ph.D., B.Sc., Pathological Laboratory, Milton Road, Harpenden, Herts. (Vice-President, 1926–1927; Council, 1912–1913, 1925–1927.)

1928 PICKLES, A., B.Sc., Imperial College of Tropical Agriculture, Trinidad, B.W. Indies.

1915 PORTER*, A., D.Sc., Department of Zoology, McGill University, Montreal, Canada.

1934 POTTER, C., B.Sc., Rothamsted Experimental Station, Harpenden, Herts.

1907 POULTON*, Prof. Sir EDWARD B., M.A., D.Sc., LL.D., F.R.S., Wykeham House, Banbury Road, Oxford. (President, 1922–1923; Vice-President, 1909–1913.)

1938 POWELL, A. K., M.Sc., Imperial College of Science, London, S.W. 7.

1919 PRAIN*, Sir DAVID, Lt.-Col., C.M.G., C.I.E., M.A., M.B., F.R.S., LL.D., F.R.S.E., V.M.H., The Well Farm, Warlingham, Surrey. (President, 1919–1921.)

1934 PRATT, A., B.Sc., Bell-Baxter School, Cupar, Fife.

1923 PRESTON, N. C., B.Sc., Harper Adams Agricultural College, Newport, Salop.

1928 RAMSBOTTOM, J., O.B.E., M.A., Dr.Sc., F.L.S., Keeper of Botany, British Museum (Natural History), Cromwell Road, London, S.W. 7.

1931 REICHERT, I., Ph.D., Chief Plant Pathologist, P.O. Box 15, Rehoboth, Palestine.

1921 RICHARDS, E. H., B.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.

1922 RICHARDS, O. W., M.A., D.Sc., 29*a*, Edith Road, London, W. 14.

1936 RILEY, N.D., Keeper of Entomology, British Museum (Natural History), London, S.W. 7.

1921 ROACH, W. A., D.Sc., A.R.C.S., D.I.C., A.I.C., East Malling Research Station, East Malling, Kent. (Council, 1935–1936.)

1914 ROBERTS, A. W. RYMER, M.A., F.R.E.S., Molteno Institute for Research in Parasitology, Cambridge.

1923 ROBINSON, D. H., B.Sc., Harper Adams Agricultural College, Newport, Salop.

1918 ROBSON, R., Institute of Agriculture, Chelmsford.

1920 Roebuck, A., N.D.A., Midland Agricultural College, Sutton Bonnington, Loughborough. (Council 1938- .)

1929 Rogers, W. S., B.A., East Malling Research Station, East Malling, Kent.

1919 Russell, Sir E. John, D.Sc., F.R.S., Director, Rothamsted Experimental Station, Harpenden, Herts. (Vice-President, 1923; Council, 1921–1923.)

1932 Russell, T. A., B.Sc., A.R.C.S., Department of Agriculture, Paget East, Bermuda.

1929 Salaman, R. N., M.D., F.R.S., Director, Potato Virus Research Station, School of Agriculture, Cambridge.

1914 Salmon, Prof. E. S., F.L.S., S.E. Agricultural College, Wye, Kent. (Council, 1916, 1924–1926.)

1935 Sampson, Miss K., M.Sc., Agricultural Buildings, University College of Wales, Aberystwyth.

1923 Samuel, G., B.Sc., Pathological Laboratory, Milton Road, Harpenden, Herts. (Council 1935–1937.)

1921 Sargent, R. H., Danescroft, Cleveland Avenue, Darlington.

1933 Schimmer, Miss F. C., M.Sc., A.R.C.S., D.I.C., Horticultural College, Swanley, Kent.

1919 Searle, G. O., B.Sc., Linen Industry Research Association, The Research Institute, Lambeg, Co. Antrim, N. Ireland.

1932 Sharga, U. S., M.Sc., Ph.D., Agricultural Dept., Etawah, U.P., India.

1938 Shaw, H., M.Sc., Ph.D., East Malling Research Station, East Malling, Kent.

1932 Sheffield, Miss F. M. L., Ph.D., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.

1936 Sian, W. E., 11 Grove Road, South Woodford, London, E. 18.

1920 Small, Prof. J., D.Sc., F.L.S., Queen's University, Belfast, N. Ireland.

1928 Small, T., M.Sc., A.R.C.S., The States Experimental Farm, Trinity, Jersey.

1920 Smith, E. Holmes, B.Sc., Botany School, Victoria University, Manchester.

1919 Smith, J, Henderson, M.B., Ch.B., B.A., Department of Plant Pathology, Rothamsted Experimental Station, Harpenden, Herts. (Treasurer, 1931- ; President, 1936–1937.)

1920 Smith, Kenneth M., D.Sc., Ph.D., F.R.S., Potato Virus Research Station, School of Agriculture, Cambridge.

1927 Smith, Prof. N. J. G., M.A., B.Sc., Ph.D., Rhodes University College, Grahamstown (Cape), S. Africa.

1913 South, F. W., M.A., 73 Fairway, Aldwick Bay Estate, Bognor Regis, Sussex.

1919 Speyer, E. R., M.A., Experimental and Research Station, Cheshunt, Herts. (Council, 1931–1933; 1937- .)

1920 Spinks, G. T., M.A., Research Station, Long Ashton, Bristol.

1920 Stapledon, Prof. R. G., C.B.E., M.A., Director, Imperial Bureau of Plant Genetics, Agricultural Buildings, Alexandra Road, Aberystwyth.

1933 Steer, W., Danelaugh, London Road, Leybourne, West Malling, Kent.

1925 Stell, F., Department of Agriculture, Port of Spain, Trinidad, British W. Indies.

1936 Steven, W. F., M.A., 110 Church Road, Richmond, Surrey.

1922 Stirrup, H. H., M.Sc., Midland Agricultural College, Sutton Bonnington, Loughborough.

1919 STONE, H., c/o Messrs Benton and Stone, Bracebridge Street, Birmingham.

1926 STOREY, H. H., M.A., Ph.D., East African Agricultural Research Station, Amani, Tanga, Tanganyika Territory, E. Africa.

1927 STOUGHTON, Prof. R. H., D.Sc., A.R.C.S., The University, Reading, Berks. (General and Botanical Secretary, 1933–1934; Botanical Secretary, 1935.)

1933 SUTTON, M. A. F., F.L.S., Sutton and Sons, Ltd., Reading, Berks.

1919 TABOR, Prof. R. J., B.Sc., Botany School, Imperial College of Science, London, S.W. 7.

1935 TARR, H. L. A., Ph.D., Fisheries Research Board, Ottawa, Canada.

1921 TATTERSFIELD, F., D.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.

1935 TAYLOR, C. B., B.S.A., Ph.D., Division of Bacteriology, Department of Agriculture, Central Experimental Farm, Ottawa, Canada.

1914 TAYLOR, H. V., O.B.E., D.Sc., A.R.C.S., Commissioner for Horticulture, Ministry of Agriculture and Fisheries, 10, Whitehall Place, London, S.W. 1. (Council, 1927–1928.)

1936 TEMPLEMAN, W. G., M.Sc., Imperial Chemical Industries, Jealott's Hill Research Station, Bracknell, Berks.

1927 THAYSEN, A. C., Ph.D., 3, Cumberland Court, 83, West Hill, S.W. 15. (Council, 1933–1935.)

1931 THOMAS, I., M.Sc., School of Agriculture, University College of N. Wales, Memorial Buildings, Bangor.

1938 THOMPSON, H. W., M.Sc., Department of Agriculture, The University, Leeds, 2.

1928 THOMPSON, W. R., F.R.S., D.Sc., Imperial Institute of Entomology, Farnham House Laboratory, Farnham Royal, Bucks. (Vice-President, 1930–1932.)

1933 THOMSON, J. R., B.Sc., School of Agriculture, Khartoum North, Sudan. Herts. (Council 1936–1938.)

1919 THORNTON, H. G., B.A., D.Sc., Rothamsted Experimental Station, Harpenden, Herts. (Council, 1936–1938.)

1933 THOROLD, C. A., B.Sc., Department of Agriculture, Nairobi, Kenya.

1934 THORPE, W. H., M.A., Ph.D., Zoological Laboratory, The Museums, Cambridge.

1927 TINCKER, M. A. H., M.A., D.Sc., R.H.S. Laboratories, Wisley, Ripley, Surrey. (Council, 1930–1932.)

1919 TROW, Principal A. H., D.Sc., F.L.S., 9, Clive Crescent, Penarth, Glamorgan.

1915 VAN DER BYL, Prof. P. A., M.A., D.Sc., F.L.S., University of Stellenbosch, Stellenbosch, Union of S. Africa.

1929 VYVYAN, M. C., M.A., East Malling Research Station, East Malling, Kent.

1920 WAKEFIELD, Miss E. M., M.A., F.L.S., Royal Botanic Gardens, Kew, Surrey.

1927 WAKELY C. T. N., B.Sc., Africa House, Kingsway, London, W.C. 2.

1932 WALDIE, J. S. L., B.Sc., N.D.A., The University, Reading, Berks.

1923 WALKDEN, H., The Raft, Derbyshire Road, Sale, Cheshire.

1936 WALKER, Miss M. M., B.A., The Horticultural College, Swanley, Kent.

1931 WALLACE, E. R., B.A., Agricultural Institute, Kirton, nr. Boston, Lincs.

1928 WALLACE, G. B., B.Sc., Ph.D., Department of Agriculture, Morogoro, Tanganyika Territory, E. Africa.

1919 WALLACE, J. C., M.C., Director, Agricultural Institute, Kirton, nr. Boston, Lincs.

Orig. WARBURTON, C., M.A., Yew Garth, Grantchester, Cambridge. (Council, 1905–1913.)
1919 WARE, W. M., D.Sc., S.E. Agricultural College, Wye, Kent. (Council, 1930–1933.)
1922 WARINGTON, Miss K., D.Sc., Rothamsted Experimental Station, Harpenden, Herts.
1930 WATSON, Mrs M. A., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
1920 WATT, A. S., Ph.D., B.A., Botany School, Cambridge.
1918 WEST, C., D.Sc., A.R.C.S., D.I.C., F.L.S., Low Temperature Research Station, Downing Street, Cambridge. (Council, 1931.)
1923 WESTON, W. A. R. DILLON, M.A., Ph.D., School of Agriculture, Cambridge.
1937 WHITE, H. L., M.A., Ph.D., D.I.C., Experimental and Research Station, Cheshunt, Herts.
1921 WHITEHEAD, T., D.Sc., A.R.C.S., University College of North Wales, Memorial Buildings, Bangor.
1937 WICKENS, G. M., B.Sc., Ph.D., Tobacco Research Station, P.B. 74 B, Salisbury, S. Rhodesia.
1937 WILKINS, W. H., M.A., D.Phil., F.L.S., Mycology Laboratory, University Department of Botany, Oxford.
1912 WILLIAMS, C. B., M.A., Sc.D., Rothamsted Experimental Station, Harpenden, Herts. (Vice-President, 1934–1935; Council, 1933–1935.)
1930 WILLIAMS, P. H., B.Sc., Experimental and Research Station, Cheshunt, Herts. (Council, 1934–1936.)
1919 WILLIS, J. C., M.A., Sc.D., F.R.S., F.L.S., c/o Empire Cotton Growing Corporation, Kings Buildings, Dean Stanley Street, London, S.W. 1.
1923 WILSON, Miss A. P., A.R.C.S., "Pencraft", Bingham Road, Radcliffe-on-Trent, Notts.
1933 WILSON, A. R., B.Sc., Ph.D., Midland Agricultural College, Sutton Bonington, Loughborough.
1914 WILSON, M., D.Sc., A.R.C.S., Royal Botanic Garden, Edinburgh.
1921 WILTSHIRE, S. P., M.A., D.Sc., Imperial Mycological Institute, Kew, Surrey. (Council, 1926–1929; 1935–1937; Vice-President 1936.)
1932 WOOD, J., A.R.C.S., D.I.C., Agricultural Institute, Kirton, nr. Boston, Lincs.
1926 WOODWARD, R. C., M.A., Imperial Chemical Industries, Jealott's Hill Research Station, Bracknell, Berks. (Council 1931–1932.)
1914 WORMALD, H., D.Sc., A.R.C.S., East Malling Research Station, East Malling, Kent. (Council 1936–1938; Vice-President, 1938.)
1936 WORSLEY, R. R. Le G., Ph.D., A.R.C.S., D.I.C., East African Agricultural Research Station, Amani, Tanganyika Territory, East Africa.
1920 WORTLEY, E. J., F.I.C., M.B.E., F.C.S., Director of Agriculture, St Anns, Port of Spain, Trinidad, British West Indies.

LAWS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

I. The Association shall be called "The Association of Applied Biologists."

II. The object of the Association shall be to promote the study and advancement of all branches of Biology with especial reference to their applied aspects.

III. The Association shall consist of Ordinary and Honorary Members.

IV. Each candidate for ordinary membership shall be a subject of the British Crown. The nomination form of each candidate for ordinary membership shall bear the signatures of two members and shall be forwarded to the Secretaries. The nomination shall be submitted to the Council and, if approved, the election of the candidate shall be recommended to the Association at the next General Meeting. For the election of any candidate two-thirds of the votes of the members present and voting shall be cast in favour of the candidate.

V. All ordinary members on first election shall pay an entrance fee of half-a-guinea. Ordinary members shall pay an annual subscription of twenty-five shillings, due on 1 January of each year, or may compound for their subscriptions by payment of a sum of twenty-five pounds.

VI. Every member elected to the Association shall receive notice to that effect from the Secretaries and shall continue a member until his written resignation shall be received by the Secretaries, or until his membership be forfeited under the laws. (A member shall be liable for the annual subscription for the year in which his resignation takes effect and, notwithstanding his resignation, shall, if he so desires, receive any subsequent publications of the Association issued during that year.)

VII. Ordinary members shall be entitled to admission to all the meetings of the Association, to vote thereat, to present papers, to take part in discussions, and to receive a copy of the Association's publications. Each member shall be entitled personally to introduce non-members to any General Meeting of the Association. But no member whose subscription is in arrears shall be entitled to vote at a General Meeting or to receive the Association's publications, nor shall any publication be sent to a new member until his entrance fee and subscription shall have been received.

The Council may remove from the roll of the Association any member whose subscription is one year or more in arrears.

VIII. Honorary Members shall be persons, not subjects of the British Crown, who have contributed to an eminent degree to the advancement of the Science of Applied Biology. They shall be recommended by a majority of the whole Council and elected in the same manner as Ordinary Members. The number of Honorary Members shall not exceed twelve and not more than two shall be elected in any one year.

Honorary Members shall each receive a copy of the Association's publication and shall not be liable for the payment of an entrance fee or annual subscription.

Their privileges shall be the same as those of Ordinary Members except that they shall not be entitled to vote at any election or meeting of the Association.

IX. The business of the Association shall be conducted by a Council consisting of a President, a Treasurer, the Secretaries, of whom there shall be two (one representing the Botanical, the other the Zoological Sections of the Association), the Editors of the Annals, of whom there shall be two, and twelve Ordinary Members. Two members of the Council shall be nominated by the President to act as Vice-Presidents.

X. All properties of the Association, both present and future, shall be deemed to be vested in the Council of the Association for the time being, in conformity with the provisions of the Literary and Scientific Institutions Act, 1854.

XI. The Council shall meet at such times as they may determine; six members shall form a quorum.

XII. The Council shall have the power to fill any vacancies among its number that may occur other than those resulting from the selection for annual retirement from the Council referred to in Law XVII.

XIII. The Council shall have power, at any of their meetings, by two-thirds of the votes of those present and voting, to recommend the removal from the roll of membership of the name of any member for the reason that in their opinion it is contrary to the interests of the Association that he shall remain a member. Such recommendation shall be submitted to the Association at the next General Meeting. For the ejection from the Association of any member two-thirds of the votes of the members present and voting shall be cast in favour of such ejection.

XIV. The Council shall appoint a Publications Committee consisting of the Editors, the Treasurer, two Ordinary Members of the Council, and two Ordinary Members of the Association, who shall be responsible for the publication of the Journal of the Association.

XV. The Council, at a meeting prior to the Annual General Meeting, shall appoint one or more Auditors to audit the Treasurer's accounts.

XVI. The Council shall purchase such books, instruments, specimens, furniture and other necessaries as may be required, pass the accounts and authorize their payment, and generally manage the affairs and administer the funds of the Association.

XVII. At a meeting prior to the Annual General Meeting the Council shall nominate a President, a Treasurer, two Secretaries, two Editors, and twelve Councillors, four of whom may not have served during the previous year, for recommendation to the Association for election in the ensuing year. Any member of the Council vacating office shall not be eligible for re-appointment as an ordinary member of Council until after the lapse of twelve months. The list as drawn up by the Council shall be sent to all members resident in Great Britain and Ireland at least four weeks before the date of the Annual General Meeting. It shall be competent for any member, on receipt of the recommendations of the Council, to add the name of a member or members of the Association to the list of candidates for election to the Council; such additional nominations, duly seconded, must be in the hands of one of the Secretaries not less than fourteen days before the Annual General Meeting.

The Secretary shall when necessary, and not less than seven days before the Annual General Meeting, issue to every member of the Association resident in Great Britain and Ireland a completed list of the proposed Officers and Council for

the year, indicating the names of the proposers and seconders of candidates other than the Council's nominees.

The election of new Officers and Council shall be conducted in the following manner:—At the Annual General Meeting each member present shall receive the list of Officers and Council proposed for the year. If no additional nominations have been received after the Council's recommendations, the list shall be put to the meeting and voted on by a show of hands and the result declared by the Chairman. If additional nominations have been received a ballot shall be taken; each member voting shall hand in person to one of the Secretaries a copy of the list on which has been indicated the names of those candidates whom the member voting desires to serve on the Council. When the ballot has been declared closed the Chairman shall appoint, from among the members present, two persons, not candidates for election, to serve as Scrutineers. In examining the lists handed in the Scrutineers shall set aside and take no account of any ballot paper which supports candidates in excess of the number provided for in Law IX, nor of any ballot paper which indicates the identity of the member voting. The Scrutineers shall report to the Chairman of the meeting the result of their scrutiny, and the Chairman, before the close of the meeting, shall announce the result of the ballot. In the case of an equality of votes for any candidates, the power of selection between them shall rest with the Chairman of the meeting and shall be exercised before announcing the result of the ballot.

XVIII. The Association shall meet at times and places to be decided by the Council.

At all Ordinary General Meetings ten shall form a quorum (see also Law XIX). All meetings shall be announced by circular addressed to each member resident in Great Britain and Ireland. At all Ordinary General Meetings the order of business shall be decided by the Chairman.

An Annual General Meeting shall, unless otherwise decided by the Council, be held on the date of the Ordinary General Meeting falling nearest to the beginning of the year.

At this Annual General Meeting the order of business shall be:

1. The reading of the minutes of the previous meeting.
2. The reading of a report of the Council on the work of the past year.
3. The statement of the Treasurer.
4. The election of Members.
5. The election of Officers and other Members of the Council.
6. Other business.

A Special General Meeting may be called to discuss or take action upon any matter affecting the interests of the Association.

A Special General Meeting shall be called either by the decision of the Council or at the request of at least ten members addressed to the Secretaries.

XIX. No new law shall be passed nor any standing law altered or added to, nor any other change in the constitution of the Association made except by a Special General Meeting of which for this purpose a fourteen days' notice must be sent to all Members resident in Great Britain and Ireland.

The requisition for such a Special General Meeting duly signed and stating in writing the laws proposed or the alteration desired, must be delivered to one of the

Secretaries, who shall within a reasonable period call such a meeting. The proposed new laws or alterations in the laws shall be printed in the circular convening the meeting.

At a Special General Meeting convened for the purpose of altering the constitution or amending the laws, fifteen shall form a quorum and no motion can be passed except by a two-thirds majority of those present and voting.

Note.

The following resolutions have been passed by the Council of the Association:

(1) That manuscripts reporting investigations dealing primarily with proprietary substances of which the composition or nature is not specified in such a way that the investigations can be repeated by other workers be not eligible for publication in the *Annals of Applied Biology*.

(2) That the mention of proprietary substances without specification of nature or composition be permissible in a manuscript provided that the particular substances are well known or standard and are only incidental in the work or contributory or subsidiary to the main theme or are used for purposes of control or comparative experiment.

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